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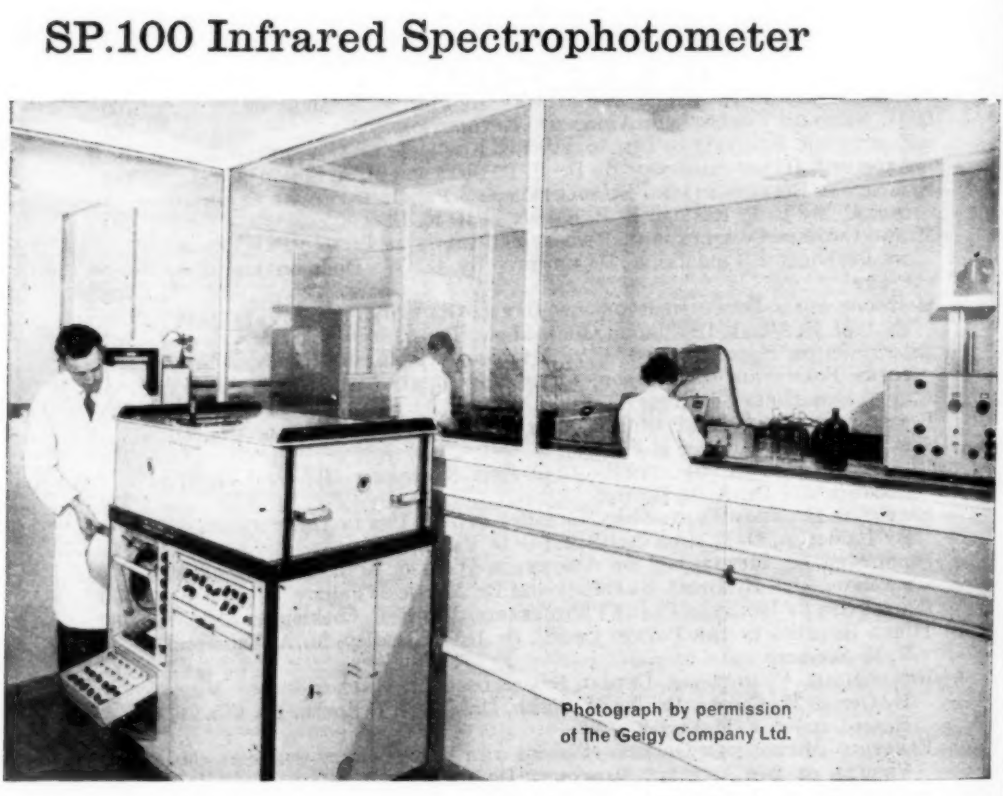

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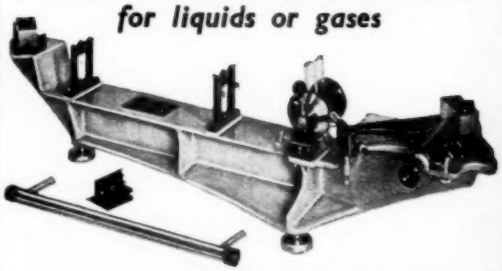

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APPRENTICESHIP AND TECHNICAL EDUCATION

THE debate in the House of Commons on apprenticeship and training, postponed from May 8, fell appropriately in the middle of Commonwealth Technical Training Week, to which reference was freely made in the debate. Both the Carr Report on the recruitment and training of young workers in industry and the later White Paper, *Better Opportunities in Technical Training* (see *Nature*, March 4, p. 701), were much in the minds of speakers in the debate, and while few new proposals were made, the debate showed the extent to which opinion on all sides has advanced in the past three years. The purpose of the Commonwealth Technical Training Week was to stimulate thought and action on technical education in all Commonwealth countries, and while the United Kingdom has no grounds for complacency in the matter, any deficiencies are the responsibility of both major political parties. On all sides of the House of Commons there are those who have sought to rouse public opinion to a sense of what is required, and have pressed constructive proposals on successive Governments, but nevertheless of 550,000 young persons who in 1960 in Great Britain entered employment, 420,000 went into unskilled jobs.

In his speech at Guildhall inaugurating the week, the Duke of Edinburgh said that he refused to believe that this is the right proportion either for the individuals concerned or for the country as a whole; and, in opening the debate in Parliament, Mr. F. Lee agreed, adding that there were many who wished to enter apprenticeship but who were denied the opportunity, and that new production methods demand new skills. A nation which does not acquire new skills condemns itself to obsolete production methods and continues to train young people in skills which have lost much of their value. The skill of many of our craftsmen is, Mr. Lee suggested, becoming outmoded, and we need to re-train many of our present craftsmen as well as to train more apprentices.

Mr. Lee, and other speakers in the debate on May 31 and in an earlier debate on apprenticeships a year earlier, on June 30, 1960, stressed the need for the trade unions to take account of these changes in technique, and while Mr. John Hare, the Minister of Labour, who followed, agreed with what Mr. Lee had said as to the importance of training, he emphasized that this is a matter for employers as well as trade unions, and that it is necessary to consider whether our present methods of training will stand the test of the working life of the boy who is being trained. He believes that the time has come for industry to have a fresh look at the content and methods of training, and suggested that the quality of training would be improved if employers made greater use of the facilities available for further education; the whole of Britain should benefit if employers made greater use of block release courses, but, particularly in Scotland, many apprentices do not get the day release recommended in apprenticeship schemes.

Mr. Hare, who rejected the charge that the Government has been inactive in the matter, referred to some progress which has been made since the Carr Report was discussed and the Industrial Training Council established, without suggesting that there is any ground for complacency. On the contrary, he agreed that the real testing time lies ahead, for the number of school-leavers will rise to 660,000 this year and to 720,000 next year. To impress this on both sides of industry, the Industrial Training Council had called a series of conferences throughout Britain, and, following these conferences, regional committees have been set up which will keep under review and encourage arrangements for recruitment and training during the peak years of the bulge. He believes that these committees will be a most valuable reinforcement to the measures already taken to encourage increased training.

The vital question, Mr. Hare insisted, is the quality of training, and this was the keynote in speeches which the Duke of Edinburgh made during Commonwealth Technical Training Week. In opening the week at Guildhall on May 29, he gave four basic reasons why every country should organize its technical training as efficiently as possible. First, the standard of intelligence, competence and workmanship in industry, commerce and agriculture controls absolutely the ability of any country to hold its own in the highly competitive business of international trade. Secondly, even in the least-developed countries, there is growing mechanization in all the public services, such as transport, communications, water, power and light. In the more developed countries, mechanization has gone much further. As a result, there is a growing need for technically competent persons capable of maintaining this increasingly complicated mechanical complex. Thirdly, technical training is essential if individuals are to make the best use of their aptitudes; and fourthly is the need for teachers, especially in technical subjects. Such teachers are not only needed in Britain. It should be obvious that the less-developed countries are less developed because the standard of technical knowledge is not very high, and the United Kingdom has the duty of providing more teachers not only for her own expansion but also for the developing countries of the Commonwealth.

One of the tasks of the week, however, the Duke of Edinburgh added, was to direct the attention of employers, trade unions and technical teachers to the need for bringing the whole complicated, confused, unco-ordinated system of apprenticeship and technical training in Britain up to date; or, where no system exists, to work out a sensible arrangement quickly. Until the Carr Report appeared, that was a controversial issue; but no feature of the debate on May 31 and the similar debate in June of last

year has been more welcome than the evidence of a disposition to re-examine this question. Commonwealth Training Week should certainly encourage this development. In the House of Commons, not only was the rigid five-year period for apprenticeship challenged as irrelevant to modern conditions, but also some constructive suggestions were advanced. Mr. John Robertson believes that apprenticeship should be considered as a continuation of full-time education and transition from class-room to workshop made less abrupt. He would put training under the control either of education authorities or of joint apprenticeship committees representing both sides of industry and the education authorities.

The latter suggestion appears to be a move towards apprenticeship to an industry rather than to a firm, a suggestion which has already found expression in Parliament and was clearly in the minds of other participants in the debate. Mr. Robertson recognized that some measure of compulsion such as day-release might be necessary, and, coming from an experienced trade union organizer, intimately associated with skilled industrial workers and well aware of their problems and outlook, his speech is surely significant. He admitted frankly that long-established customs and practices in industry may need to be changed, and pointed specifically to the lack of knowledge of the numbers and categories of apprenticeships and to the widespread support on both sides of industry for a national register of apprentices; he also suggested in this connexion that raising the school-leaving age might be helpful.

Two other constructive speeches in the debate may be mentioned. Mr. R. L. Mawby looks for more mobility of skilled labour and advocated re-training of skilled workers to this end. While he was less concerned about the five-year period of apprenticeship—though he urged the need for greater flexibility—he was concerned about the quality of the training given during those years and rightly urged that this is what really matters. The importance of skill is increasing, and industry should see that the right incentive is given to new apprentices to acquire new skills. He seemed more hopeful than most speakers about the Industrial Training Council, if it is adequately strengthened and supported.

Mr. R. E. Prentice, whose speech was particularly welcomed by the Parliamentary Secretary to the Minister of Labour, Mr. P. J. M. Thomas, in concluding the debate, was concerned especially with the challenge of the bulge and the opportunity it affords, but urged that much more attention must be given to the question of what constitutes a proper apprenticeship or proper industrial training in the second half of this century. He began, like most speakers, by challenging the rigidity of a five-year period for apprenticeship, and urged that more attention must be given to standards of training and that we need to train many more people to handle a wide range of skilled work.

A feature of the debate was, in fact, this recognition of the need for greater flexibility, but Mr. Prentice made once again his suggestion for some kind of differential training levy rather than the pay-roll ta proposed in the Budget last April, which he though is likely to discourage apprenticeship schemes. Th differential training levy, as operated in France fo many years, could penalize the firm which poache trained workers and did not train its own, and assis the firm which did its full share or more of training He was doubtful as to the value of an apprenticeshi council, but thought that the Industrial Trainin Council needs more staff and resources.

The Parliamentary Secretary welcomed the em phasis on flexibility and stressed that the key to th situation at the craft-level lies in the willingness o employers and unions to increase the intake o apprentices. He also said that the establishment o a national craft apprenticeship system is a long-ter process; he did not comment on this proposal for differential training levy. He welcomed many of th suggestions, however, and particularly the reference made to the relation between training in technica colleges and industrial training, to which Miss J. M Quennell had directed particular attention; he urge the need to consider the strains and stresses whic the situation is placing on technical colleges i Britain and the importance of further inquiry int the field of technical education and industry's futur requirements for technicians. But he was as firm a Mr. Hare that training should not be completel divorced from production and that industry mus accept its training reponsibilities. The Government efforts should be designed to reinforce and not sup plant industry's own efforts.

When the pay-roll clause in the Finance Bill wa debated in the House of Commons on June 13, Mr Prentice did not press his plea for a training levy though he again referred to it; but he strongly urge that surcharges should not be payable in respect o employees under eighteen. Mr. R. E. Carr also urge that the pay-roll tax should be dropped. But i replying for the Government, the Financial Secretar to the Treasury, Sir Edward Boyle, would go n further than admit that if the surcharge came to b imposed, there would be a strong case for specia treatment for boys and girls under eighteen. Si Edward declined to enter into a firm commitment t provide partial or total relief for boys and girl under eighteen, but promised that the argument advanced would be kept in mind.

Miss Quennell, who was among those who cha lenged the appropriateness of a five-year apprentice ship, suggested that the technical colleges could pla a greater part. First-year apprenticeship courses ar indeed being introduced in some technical college and the proposals of the White Paper on technica education are intended to help generally here, no only by improving selection and broadening th content of courses, reducing wastage and filling i gaps in the existing arrangement, but also especiall by stimulating co-operation between the education authorities and both sides of industry. The implica tion of the policy set forth in the White Paper and o the new routes to further education which it provides were indeed discussed in a series of four paper

presented at a conference on April 19 arranged by the British Association for Commercial and Industrial Education*.

In the first of these, Mr. G. E. Manning stressed the importance of good teachers in order to make full use of the potentialities of the secondary modern schools and the technical schools and colleges of Britain. Here the questions of salary and status cannot be ignored. Prof. Boris Ford directed attention to the danger that an elaborate system of regional examining boards might destroy the individuality and diversity of our modern schools as well as depress the majority of children who are not good at working at subject examinations. It is important, he said, to encourage the maximum opportunity for independent experiment, and he thought we should work towards a national policy of an award for every school-leaver based on comprehensive assessment, and supported (where relevant) by regional certificates awarded on the basis of local examinations moderated regionally. He also insisted that the education of pupils of average or less than average ability between the ages of thirteen and sixteen, which the Minister has now asked the Central Advisory Council to consider, is the most difficult and worrying segment of the educational system of Britain.

Dr. Ethel Venables, who looked at the proposals from an educational point of view, said that the major problem of educating for industry is not one of selection or teaching methods, important as these are, but of how we could encourage the desire to learn. She suggested that the real way to tackle this problem is to recognize that able young people are a national asset, and that they cannot be educated adequately if a sense of purpose is lacking; society in general and industry (including the trade unions in particular) must be ready to reward achievement at all levels. The nature of the goal would vary throughout industry, but much more time must be devoted to general and social studies.

Mr. F. H. Perkins, speaking from the industrial and commercial point of view, stressed the importance of enabling a transfer from a national certificate to a craft course to be made as early and smoothly as possible and also of much closer co-operation between the technical colleges and industry. He, too, referred to the difficult problem of a sufficient supply of teachers, some of whom must come from industry, and to the need for flexibility if operative courses, following suitable selection, were to give useful service to industry. The need for clear thinking and for careful definition of terms was obvious in Mr. Perkins's paper, and some of the speeches in Parliament showed that even now the functions of the technical colleges and of the colleges of technology are liable to be confused.

Nevertheless, the debate on May 31 demonstrated that if, in his appeal for a fresh look at the system of apprenticeship in Britain, the Duke of Edinburgh was not exactly pushing at an open door, there is now a good deal of support for review. Commonwealth Technical Training Week could well go some way towards achieving its aim of getting industry to examine and improve its schemes of training, recruitment and allocation. If employers and trade unions can be induced to take a fresh look at this question of training, putting aside out-of-date attitudes and thinking only in terms of what is relevant in the second half of the twentieth century, it will have been well worth while. The publicity it has received should contribute to that end, for, as the Parliamentary Secretary to the Ministry of Labour rightly said, an interested and informed public opinion is essential; without it, the necessary measures are unlikely to be initiated or effectively implemented. Commonwealth Technical Training Week is at most only a beginning.

It is important, therefore, that full use should be made of the wider interest it has created and the clear public understanding of some of the issues that are involved. There may now perhaps be the less danger that necessary inquiries into methods of teaching already under way will tend to discourage the students themselves, though it is to be hoped that too much emphasis will not be placed on this aspect while the policies of the White Paper are being given effect. The new group in the Oxford University Department of Education which is studying the reasons why comparatively fewer students read for technological qualifications in Britain than in the U.S.S.R. or the United States, the sub-committee of the National Advisory Committee on Education for Industry and Commerce which is examining the organization of sandwich courses, and the investigation at Birmingham under Dr. T. Lupton, of the College of Advanced Technology, with the aid of a Nuffield grant, of the quality of industrial and college training, should provide some of the new knowledge for which Miss Quennell, for example, pleaded; but such investigations must not be allowed to give an impression that so much is wrong with existing teaching that it is not worth while for a student to enrol in a technical institute. Incidentally, in a written answer in the House of Commons on June 15, Sir David Eccles said that his Department is establishing a new branch to give emphasis to educational research generally.

Again, little good is likely to come of the meeting of the Minister of Education with representatives of English local authorities to discuss the status of the colleges of advanced technology unless a well-informed public opinion is available to support whatever decisions may be reached. For the Minister to succeed in making these colleges independent, on university lines, would not of itself suffice to solve all the many difficult problems, and indeed might raise fresh issues in regard to technical education because the regional principle has been insufficiently regarded in creating these problems. Indeed, one major asset of Commonwealth Technical Training Week has been the way in which it has directed attention to technical education as a whole, at all

* British Association for Commercial and Industrial Education. New Routes to Further Education: Papers presented at the B.A.C.I.E. Spring Conference, Senate House, University of London, 19 April, 1961. Pp. ii+31. (London: British Association for Commercial and Industrial Education, 1961.)

levels, from that of the university department and of the college of technology to that of the secondary modern and technical school. Furthermore, it should have indicated to many how important it is that Britain should play her full part in expanding technical training in the Commonwealth as a whole. It is not sufficient to fill the gaps in the existing system in Britain, to improve the quality of the training given and to extend its scale: we must both provide a reasonable number of places for overseas students in the colleges and universities of Britain, and also supply an increasing number of qualified and enthusiastic teachers for overseas, especially in the less-developed territories.

That is sufficient reason for urging much closer co-operation between the Ministry of Education, industry, the universities and others concerned with technical training. Firm assurances were given in the House of Commons as to the co-operation between the Ministry of Labour and the Ministry of Education, and Mr. Robert Carr gave convincing reasons last June why technical training in industry should not be made a responsibility of the Ministry of Education, while at the same time he urged as strongly as Mr. Austen Albu, who made the suggestion, the need for closer co-operation between the two Ministries. The Minister for Science, however, is also vitally concerned, through his responsibilities for the Department of Scientific and Industrial Research alone, as is the Chancellor of the Exchequer, through his responsibility for the University Grants Committee.

It will be recalled that the Carr Committee itself recommended greater flexibility in the age of entry to apprenticeship as well as that opportunity for change of employment should not be limited on grounds of age. The strong support which has been indicated for giving the first year's training in the school rather than in the workshop, and the critical look at the quality of teaching in the workshop which was noticeable in the House of Commons, at least as much from the trade union side as from anywhere else, show the extent to which public opinion has moved since the Carr Report appeared, and how largely Lady Gertrude Williams's criteria are becoming accepted: the first—an adequate supply of training vacancies—was thoroughly discussed during the Commonwealth Technical Training Week. The importance of spreading the costs of training more justly over all firms benefiting from the employment of adult skilled men, of ensuring that men capable of learning to do a job are not permanently excluded simply because they missed the opportunity to train when they were sixteen, and of improving the quality of teaching in the workshop, were fully recognized in the recent debate. On exactly how best to achieve these ends there is still room for discussion and inquiry, and perhaps more especially on the further criteria that theoretical instruction should be graded to suit the capacities of pupils. The scientist and technologist must bear a large responsibility for seeing that matters are not allowed to drift and that the co-operation and new outlook made evident during Commonwealth Technical Training Week are maintained. It is welcome evidence of the acceptance of such responsibility that Mr. G. C. Eltenton should devote such a large part of his presidential address on May 23 to the Society of Instrument Technology to consideration of the education and training of the technologist and technician.

WILLIAM HARVEY

William Harvey : Englishman, 1578–1657
By Prof. Kenneth J. Franklin. Pp. 151+5 plates. (London: Macgibbon and Kee, 1961.) 18*s*. net.

FOR three hundred years, Harveian Orations have been delivered annually to the Royal College of Physicians, and several 'orators' have observed that there is little new to be said about Harvey's life and work. Yet within the past few years fresh information has been forthcoming. The discovery of the Harveian Oration for 1662, ascribed to Sir Charles Scarborough, is a pupil's testimony to Harvey's ideals, knowledge and inquiring mind[1]. Dr. Richard Hunter and Dr. Ida Macalpine have assembled data about Harvey's knowledge of neurology and psychiatry[2]. This aspect has been further extended by Mrs. Gweneth Whitteridge, who with great skill, ingenuity and patience has deciphered Harvey's notes on *De motu locali animalium* (1627), being his views on muscular contraction and its control by the brain[3]. Lastly, Sir Russell Brain, in his Harveian Oration for 1959, has given a comprehensive account of "William Harvey, Neurologist", not suggesting that he contributed anything new to neurology, but illuminating him in Sir Thomas Browne's phrase as the "ocular philosopher", "a great observer of man and of nature, and a thinker whose intuition saw when traditional ideas were outmoded, even though he did not always know how they were to be replaced".

A letter of Bishop Brian Duppa to Sir Justinian Isham[5] relates that Harvey paid attention to dietetics and nutrition and would sometimes fast two days together; that the bishop was with him the day before he died; and that Harvey's life "went out like a spark without any violence or noise at all".

In respect of all this and further additional information, a new life of Harvey was desirable. Prof. Franklin, who has already rendered great service to Harvey's memory by his new English translations of *De motu cordis* and of *De circulatione sanguinis*, the two anatomical essays addressed by Harvey in 1649 to Jean Riolan, jun., has now written Harvey's biography.

In the first place, the author reviews earlier speculations on the circulation, and demonstrates that Ibn-an-Nafis (about 1210–88), a learned doctor from Damascus[6], only foreshadowed to some extent the pulmonary or lesser circulation; Servetus discovered it, but this received little attention. The opinions of Columbus, Vesalius, Botallus, Cæsalpinus and Fabricius, who noted the valves in veins, are also discussed. Prof. Franklin's conclusion is that the observations and opinions of the pre-Harveian "circulators in no way diminish Harvey's fame as the independent discoverer of the circulation".

Harvey's life and work are next described, and attention is paid to the importance of Harvey's second great work, *De generatione animalium*. This would have been even more valuable had it not been for the pillage and destruction of Harvey's notes and

papers—including a volume on the generation of insects—in his lodgings in Whitehall by the soldiers of the Parliament.

Prof. Franklin writes concisely and lucidly with scholarship and literary grace. The book is well illustrated and produced.

[1] Payne, L. M., *J. Hist. Med.*, **12**, 158 (1957).
[2] Hunter, R., and Macalpine, I., *J. Hist. Med.*, **12**, 126 (1957).
[3] *Harvey De motu locali animalium*, edited, translated and introduced by Gweneth Whitteridge (Cambridge, 1958).
[4] Brain, W. Russell, "William Harvey, Neurologist", *Brit. Med. J.*, ii, 899 (1959).
[5] Isham, G., *The Correspondence of Bishop Brian Duppa and Sir Justinian Isham, 1650–1660*. Northamptonshire Record Society, **17**, for 1950–51, Letter LXXXIX (1955). See also MacNalty, A. S., "William Harvey; His Influence on Public Health", *J. Roy. Soc. of Health*, **77**, 330 (1957).
[6] Meyerhof, M., "Ibn-an-Nafis (XIII cent.) and his Theory of the lesser Circulation", *Isis*, **23**, 100 (1935).

SOUNDS USED BY ANIMALS

Animal Sounds and Communication

Edited by W. E. Lanyon and W. N. Tavolga. Pp. xiii+443+1 12-in. long playing record. (Washington, D.C.: American Institute of Biological Sciences, 1960.) 9.50 dollars.

THE critical and analytical study of the sounds used by animals in communication is a relatively new branch of biology. The reason for this is that only in the past fifteen or twenty years have recording and analytical techniques been developed far enough for the analysis of the extremely complex sound signals which animals use. Moreover, as the technology of microphones, amplifiers and tape recorders improves (as it does year by year) new possibilities for the study and understanding of animal vocalizations emerge.

The present book, edited by Drs. Lanyon and Tavolga, constitutes a valuable and stimulating guide to those interested in this field. There is an introduction by J. T. Emlen, jun., followed by five chapters, each by a different author, these being largely a transcript of the papers presented at a symposium of the American Institute of Biological Sciences, 1958. Since the vast majority of work on animal sounds has so far dealt with birds, this volume is naturally still rather over-weighted on the bird side, but it is noteworthy for three chapters on other groups—one dealing with sound communication in Orthoptera and Cicadidae, by R. T. Alexander; another on sound production and underwater communication in fishes, by W. N. Tavolga; and a third on the influence of sound on the behaviour of amphibians and reptiles, by C. M. Bogart.

All these are valuable interim summaries in rapidly expanding fields. The book starts with a useful though rather too elementary discussion of techniques by P. P. Kellogg. D. J. Borror's chapter on the analysis of animal sounds is also rather slight and elementary for 1960, but it must be remembered that it was written before August 1958.

W. E. Lanyon's chapter on the ontogeny of vocalizations in birds, and that by P. Marler on bird songs and mate selection are both admirable and forward-looking contributions, suggesting new experiments and new lines of thought. N. E. Collias contributes a very stimulating chapter in which he sets forth an ecological and functional classification of animal sounds, based on wide knowledge and reading. He suggests that the various acoustic signals of animals fall readily into ecological and functional categories related to: (1) food, (2) predators, (3) sexual behaviour and related fighting, (4) parent–young interrelations and (5) aggregations and group movements. He points out that the same sound may have more than one specific use, depending on ecological context, and sounds with similar functions, even when produced by different mechanisms and different animals, are frequently characterized by certain common properties in their physical structure. The book concludes with a chapter by Charles F. Hockett devoted to logical consideration and the study of animal communication. This is a helpful contribution, but it is perhaps written rather too largely from the point of view of a student of human language.

A most important and valuable feature of the book as a whole is that a 12-in. long-playing record is issued with it. This provides precise audio-illustrations of the chief points raised by the seven main chapters and brings to life many of the diagrams. As a result the book is immensely more valuable and intelligible than it otherwise would have been. It is to be hoped that the practice of issuing records with books on this and similar subjects will be widely adopted.

W. H. THORPE

ANALYSIS OF ORGANIC COMPOUNDS

Organic Analysis

Vol. 4. Edited by John Mitchell, Jr., I. M. Kolthoff, E. S. Proskauer, and A. Weissberger. Pp. vii+429. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.) 102*s*.

VOLUME 4 of this well-known series of publications on organic analysis gives further authoritative information regarding the application of the newer instrumental techniques and procedures.

H. Foster presents a detailed account of the principles and usages of nuclear magnetic resonance spectroscopy, and comments also on the limitations of the method. The basic principles utilized in interpreting high-resolution nuclear magnetic resonance spectra are discussed for the case of pure compounds, for example, ethanol, and also for hydrocarbon mixtures. Molecular association, gem splitting, chelation, geometrical isomerism are all illustrated by reference to typical resonance spectra; the effect of the too often ubiquitous presence of water impurity is commented on, and nuclear magnetic resonance investigations on a number of complex substances, for example, Feist's acid, steroids, alkaloids, etc., are detailed.

Nogare and Safranski have provided a most comprehensive review of the applications of gas-liquid chromatography. Theoretical principles, modern apparatus and techniques, detectors, etc., are described in great detail, and considerable reference is made to many varied and important investigations. Programmed temperature and high-temperature gas chromatography receive special treatment.

One rather unique chapter by Krc deals with crystallographic methods, and indicates the valuable information which is yielded by a combined microscopical and X-ray diffraction examination of a given substance. The value of morphological studies of polymorphic modifications, optical properties, fusion behaviour, etc., are all clearly illustrated. The experi-

XUM

mental pitfalls associated with the application of the powder X-ray diffraction technique to the identification of organic crystalline materials are indicated. The chapter contains an excellent survey of fundamental crystal optics, morphology, use of Miller indices, etc.

Neilands contributes a welcome review chapter on enzymes, their availability and their varied analytical and clinical usages. He supplies a valuable bibliography of specific applications, for example, estimation of ethanol in blood, detection of peroxides, analysis of specified amino-acids, etc.

The wide use of organic peroxides and ozonides by many industries has resulted in the production of a timely review by Martin on the determination of these occasionally hazardous reagents. The adventitious formation of peroxides in ether solvents is discussed, and the use of an activated alumina column for solvent desensitization and purification is worthy of note.

Iodometric, colorimetric, polarographic, infra-red and other related techniques for peroxide determination are described in reasonable detail, and some attention is paid to the estimation of peroxide mixtures using chromatographic or counter-current distribution separation procedures. Analytical interferences produced by sulphur compounds, conjugated diolefins, aldehydes, etc., are defined: the polarographic method of analysis is considered the most widely applicable method for peroxides generally.

The present volume ends with a chapter by Ke on the applications of differential thermal analysis to the examination of high polymers. The apparatus is described fully, and the thermograms are interpreted in terms of conventional thermodynamic parameters. Applications of this powerful technique to work on synthetic and natural polymers are clearly and extensively demonstrated.

This new volume of the *Organic Analysis* series will undoubtedly be as eulogistically received as were its predecessors. It presents, in a small compass, a considerable amount of information on the theoretical and practical aspects of the six important subjects reviewed, and provides material which will undoubtedly be of the greatest interest to the industrial chemist, the research worker and the academic student.

D. T. Lewis

STILL MORE NUCLEIC ACIDS

The Nucleic Acids

Vol. 3. Edited by Erwin Chargaff and J. N. Davidson. Pp. xvi + 588. (New York : Academic Press, Inc. ; London : Academic Press, Inc. (London), Ltd., 1960.) 18 dollars.

WHEN the first two volumes of this book were published, it was evident to the editors that the research on this subject was expanding rapidly, but even they did not anticipate the explosion which has since followed. In an attempt to bring their treatise up to date, they have now issued Volume 3, which has as its object the covering of certain aspects of the subject which had been neglected in the original volumes, or which have recently assumed particular importance. The editors should be congratulated on their decision to take this course, and the third volume of this series will certainly prove a valuable addition to many libraries. They might, however, consider at this time whether it would not be more useful to begin a new series, similar to *Advances in Protein Chemistry*, which would provide definitive articles on recent aspects of the subject and not involve so much delay in publication, rather than to continue this series. It is certainly unlikely that the compiling of a comprehensive and up-to-date text-book on nucleic acids will be feasible for many years.

The new volume is a sort of compromise between a text-book and a review. All the contributors are actively engaged in research on the subjects covered in their contributions, and while this certainly makes for authoritative articles, there is the concomitant risk of the authors giving a one-sided view of the subject. For example, someone reading Schuster's article on virus ribonucleic acids might get the impression that research on this subject began about 1954, whereas the pioneering work of Bawden and Pirie, who first discovered ribonucleic acids in a number of plant viruses some twenty-five years ago, is not referred to, though some of their later work is. Max Schlesinger, a neglected pioneer if ever there was one, is not even mentioned in an otherwise admirable chapter on bacteriophage nucleic acids written by Sinsheimer, though Schlesinger was not only the first person to purify a bacteriophage but was also the first person to identify nucleic acid in any virus. Sinsheimer devotes tantalizingly little space in this article to his own work on the single-stranded deoxyribonucleic acid of the bacteriophage $\varphi X174$, which demonstrates quite convincingly that complementary filaments of deoxyribonucleic acid are not essential for the transfer of genetic information.

Sadron's contribution is largely devoted to light-scattering and hydrodynamical methods of investigating nucleic acids which do not have enough resolving power for polydisperse solutions. However, some of the problems which he poses have since been solved by the elegant methods of Meselson and his colleagues, which unfortunately are too recent to have been included in this chapter, though they are touched on elsewhere. The photochemistry of nucleic acids is covered in considerable detail by Shugar, who also provides a useful list of recommended volumes for extra reading. Khorana reviews both the chemical and enzymic synthesis of polynucleotides, a subject to which he has contributed so much, both directly and by his synthesis of substrates, but surprisingly he omits mention of the enzymic synthesis of small polynucleotides by the reversal of the action of ribonucleases.

The chapter by Hoagland on nucleic acids and their involvement in protein synthesis is most welcome as an authoritative review of this important work, much of which has come from his laboratory, while Gros describes related happenings in bacteria.

The effects of 'antimetabolites' on nucleic acid metabolism, which a few years ago gave so much hope, unfortunately unrealized, of a rational approach to the control of many serious diseases, are covered by Handschuhmacher and Welch. Unexpectedly, this work has made a real contribution to our knowledge of nucleic acid metabolism which could scarcely have been obtained by other means.

A compendium of the analyses of microbial nucleic acids is given by Belozersky and Spirin (it is most encouraging to see a contribution from the U.S.S.R.), and the biosynthesis of purine and pyrimidine nucleotides is covered by Buchanan and Crosbie respectively. Finally, Lajtha gives a short review of the effects of ionizing radiations on nucleic acid metabolism.

Roy Markham

KENNAWAY AND THE CARCINOGENS*

By Prof. ANTOINE LACASSAGNE
Institut du Radium, Paris

I FIRST met Ernest Kennaway in 1923 at the Cancer Hospital in London when he was working on the isolation of the active carcinogenic principles in tar. In the following year Kennaway began to publish the results of the researches; the importance had escaped me a year earlier, yet the discovery of a 'carcinogenesis factor', by the pyrolysis of various organic substances, was to prove one of the most fertile in the whole of cancer research. It supplied the solution to a riddle which for more than 150 years had puzzled clinicians confronted with 'industrial cancers', but unable to understand how they arose. It opened the important chapter of chemical carcinogenesis which was to be written over the ensuing years by Kennaway himself (as director of the Research Institute of the Cancer Hospital and professor of experimental pathology) and the team of brilliant co-workers with whom he had surrounded himself. Through this work, organic chemistry was to be enriched by hundreds of new substances, and the way opened to the prophylaxis of industrial cancers.

During this time my own attention had been directed towards the study of carcinogenesis by endogenous chemical substances, that is to say, by certain steroid hormones. The structural relationship of these substances with the polycyclic hydrocarbons, the carcinogenicity of which had been demonstrated by the workers at the Cancer Hospital, had finally brought home to me the outstanding importance of Kennaway's discovery. But when I came to spend some days in his company at Oxford, in 1950, he was already gravely ill. We were among the twenty or so participants in a colloquium on the geographical pathology of cancer, held during July 29–August 4, following the International Cancer Congress in Paris and the International Radiology Congress in London. His wife was also present, sustaining the sick man with that affectionate companionship and scientific collaboration which she had lavished on him for thirty years, combined with the tenderness of a sister of mercy. Thanks to her, he retained to the end of his life what for a man like Kennaway was a true consolation—the ability to continue his work.

Great scientific discoveries come about by two means. Some are born of almost magical intuition and good fortune, suddenly laying bare a whole unsuspected realm of Nature. Others are reached by patient work and devoted attention to a particular problem. Kennaway's discovery was of the latter kind.

The observation made at the end of the eighteenth century by the British surgeon, Percivall Pott, of a relationship between the development of certain cutaneous cancers and irritation of the skin by the combustion products of coal tar, had been followed by others. Experimental work on the suspected carcinogenicity of tar, pitch and paraffin was started at the end of the nineteenth century. Not until 1915, however, was a positive and conclusive result obtained, by the sensational discovery by Yamagiwa and Ichikawa. Work was at once started in various laboratories the world over to follow up their results. It was recognized that the mouse was the animal of choice, the most convincing results being obtained in this mammal in the shortest time. The lack of conformity in the results, however, led to the observation that the activity of tars varied according to their origin. Opinions differed as to which of the numerous constituents of coal tar might be responsible for its carcinogenic activity, suspicion being directed against arsenic, the phenols, the pyrrols, aniline, benzidine, anthracene and others. A first pointer came with the recognition that only the highest boiling fractions, distilling at temperatures above 300°, possess carcinogenic activity.

It was to the unravelling of this tangled skein that Kennaway had set himself. His patient and methodical work was to supply a thread which, a few years later, was to lead the workers at the Cancer Hospital, through a rapid succession of discoveries, to write one of the fairest pages in the history of science.

Having observed that compounds distilling at a very high temperature were for the most part hydrocarbons, he had the idea of making a synthetic tar, starting from a very simple organic substance consisting only of hydrogen and carbon. A pyrolysis product of isoprene was tested on the skin of a mouse, with a positive result, and Kennaway's well-reasoned hypothesis was confirmed[1]. Actually, he obtained a higher proportion of tumours than with ordinary coal tars.

In 1925, Kennaway confirmed the results which he had published the previous year, this time using acetylene. The activity of acetylene tars varied according to the temperature at which they were prepared[2]. Other synthetic tars prepared from different organic substances, both animal and vegetable, also proved carcinogenic. In 1930, Kennaway published an even more important observation[3], starting this time from a tetrahydrogenated derivative of naphthalene, tetraline. Under the action of aluminium chloride this pure hydrocarbon acquires new properties, being in fact transformed into carcinogenic compounds. Since the work of Berthelot in 1864[4] it had been known that the pyrolysis of acetylene gives aromatic hydrocarbons by the cyclization of acyclic hydrocarbons. It therefore seemed probable that carcinogenic polycyclic hydrocarbons had in fact been produced in the earlier experiments also.

This hypothesis was confirmed when, in collaboration with Hieger, Kennaway[5] found that carcinogenic tars, whether natural or synthetic, gave a common fluorescence spectrum (three bands at 4000, 4180 and 4400 Å.) to which Mayneord had already directed attention. The fact that a tetracyclic hydrocarbon, 1,2-benzanthracene, had a rather similar spectrum, pointed to another line of inquiry in the search for the causal agent. This was justified when, among the polycyclic hydrocarbons which these authors prepared synthetically, they found one, 1,2:5,6

* Substance of the first Sir Ernest Kennaway Memorial Lecture, delivered at the Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, London, on June 12.

1,2 : 5,6-Dibenzanthracene

(I)

dibenzanthracene (I), which tested on mouse skin produced epitheliomas[6]. The way was open for fresh efforts on the part of the team at the Cancer Hospital, which had now been joined by J. W. Cook.

In 1932, Cook, in collaboration with Hewett and Hieger, had succeeded in isolating from coal tar a pentacyclic hydrocarbon, 3,4-benzpyrene (II), which

3,4-Benzpyrene

(II)

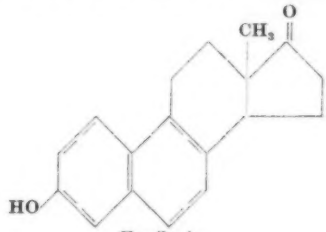
proved to be a much more potent carcinogen than the others previously synthesized[7]. Eight years of methodical work had thus supplied a solution to the problem posed a century and a half earlier.

3,4-benzpyrene seems to be the carcinogenic hydrocarbon produced most frequently and most abundantly in soot from coal fires, in the treatment of mineral oils, and, in certain circumstances, from the combustion of petroleum—all substances derived from the luxuriant vegetable and animal matter laid down during the primary era. 3,4-benzpyrene is also the principal hydrocarbon in cigarette smoke and in the vitiated air of industrial cities. It will be seen that the discoveries of which Kennaway was the author or initiator exercise a continuing repercussion on public life to-day.

They had an immediate influence on the trend of work in all the laboratories concerned with cancer research. While the team at the Cancer Hospital was widening the field of chemical carcinogens, demonstrating the induction of various types of cancer by a very large number of aromatic polycyclic hydrocarbons of differing structure (derivatives of anthracene and phenanthrene), similar teams of chemists and biologists were being formed in the United States, in Germany, Belgium, France, Japan, Norway, Switzerland and elsewhere. In a few years, numerous papers had been published on this subject and many new facts established. The time had come to classify these new data, and in 1937 [8], 1939 [9] and 1940 [10] the team at the Cancer Hospital published invaluable monographs analysing the principal papers and furnishing 877 references to earlier publications.

Since the induction of tumours by chemical means had now been amply demonstrated, it seemed that an understanding of the mechanism of carcinogenesis should soon lie within our reach. The relationship between the molecular structure and carcinogenic activity of chemical compounds was clear. The skeleton of the majority of them contains the phenanthrene ring system; oncogenic activity can be produced, enhanced, weakened or destroyed by simple substitutions on the molecule. It became possible to judge the carcinogenic potency of a given molecule simply from the ring configuration and the position of the substituents. For example, in 1933, Cook[11] predicted the carcinogenicity of a substance which had just been synthesized in Germany, and in the following year he demonstrated this experimentally, in collaboration with Haslewood[12]. The methylcholanthrene (see IV) molecule contains the phenanthrene structure common to numerous compounds the biological activity of which is nevertheless very dissimilar, such as alkaloids, certain cardiac poisons, vitamin D, steroid hormones and bile acids.

The similarity between the skeletons of the carcinogenic hydrocarbons and that of equilenin (III

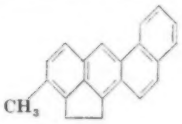


Equilenin

(III)

(a sex hormone discovered by André Girard *et al.* in 1932 in the urine of pregnant mares[13]) did not escape the attention of the research workers in Great Britain. In 1933, Cook and Dodds[14] published their finding of slight, but characteristic, œstrogenic activity of hydrocarbons such as 3,4-benzpyrene and 5,6-cyclopenteno-1,2-benzanthracene.

Was it not therefore conceivable that certain normal metabolites, of steroid structure, might be transformed into carcinogenic compounds in the body itself, as a result of one or more errors of metabolism? It was in fact not long before a new aromatic hydrocarbon, 3-methylcholanthrene (IV) was obtained and



3-Methylcholanthrene

(IV)

found to be even more active than 3,4-benzpyrene: it was produced synthetically from a bile acid, deoxycholic acid (see VIII) by dehydrogenation processes of which the organism is, however, known to be capable.

Kennaway at once perceived the connexion between this finding and an experiment of which he had published the results in 1928 with Sampson[15], and which had yielded tumours using the combustion products of cholesterol. The phenanthrene nucleus is also present in the cholesterol molecule, and it seems that steroid hormones, like bile acids, are built up from this molecule. Kennaway therefore, with his wife and Cook[16], put in hand experiments to demonstrate the carcinogenic activity of deoxycholic acid. It was two years before a single epithelioma was produced in the skin of a mouse; but subcutaneous injection produced several sarcomata *in situ*.

It was in this field of the relationship between carcinogens and hormones that collaboration started between the Research Institute of the Cancer Hospital (shortly to be named the Chester Beatty Research

Institute) and the Institut du Radium in Paris. The work which had been carried out in my laboratory since 1932 on the role of the steroid hormones in tumour induction probably influenced the workers at the Cancer Hospital in their new line of research. It is certain that the most recent publications on the part of these workers caused the line of research at the Institut du Radium to branch in the direction of the study of carcinogenesis by chemical agents.

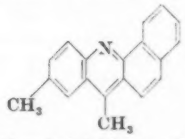
Meanwhile, Burrows, with his skill in histopathology, had proved a most valuable addition to the team at the Cancer Hospital studying induction of cancer by hydrocarbons. From 1935 onwards, he began to specialize in the study of hormonal cancers, and here too his contribution was invaluable. The recruitment of Horning to the Department enriched and accelerated its development. Henceforward I paid frequent fruitful visits to the Chester Beatty, and these two scientists became my firm friends; I cannot recall our cordial relationship without emotion.

I myself meantime had the good fortune to attract to my department a first-class organic chemist, Buu-Hoï. In collaboration with him, I was able to penetrate the vast field of exploration opened up by Kennaway, and to extend the investigations on the mechanism of carcinogenesis which I had pursued first with radiation cancers and later with hormonal cancers. I hope it will not be thought out of place if I now speak a little about the work we had been doing in Paris, which stemmed directly from Kennaway's discoveries.

It was inevitable that we should have to come to compare the action of the carcinogenic hydrocarbons with that of drugs, partly because of the close relationship between carcinogenicity and molecular structure demonstrated by Kennaway, and partly from the idea that the production of radiation cancers might be due to the probable attack on particular targets formed by intracellular organelles. In 1942 we conceived the idea of looking for proof along the lines of Ehrlich's postulate: *corpora non agunt nisi fixata*, already well established in chemotherapy. Thus the classic example of sulphanilamide and *p*-aminobenzoic acid had proved that the fixation of a pharmacodynamic agent on the cell may be disturbed by the presence of a molecule of similar structure. Using pairs of molecules consisting of a polycyclic carcinogen and an inactive or weakly active molecule, acting simultaneously on the skin of the mouse, Buu-Hoï, Rudali and I[17] found that such an antagonism between two hydrocarbons did in fact exist. The most reasonable explanation of the phenomenon seemed to us to be competition between two molecules to attach themselves to the organelle or organelles governing growth and cell division.

This fact has been more or less fully confirmed by several authors. American workers have even shown that certain hydrocarbons, such as methylcholanthrene, inhibit the production of cancer of the liver by carcinogens of very different structure, of the azo group. Two of the members of the active Madison school, the Millers, who have done so much to elucidate the problem of experimental liver cancer, have put forward a very tempting explanation of this observation[18]. With their co-workers, they were able to show that this fixation occurs at the level of certain cellular proteins[19], and thus to confirm that azo carcinogens behave in the same way as pharmacodynamic agents. It has since been found that, in the case of skin cancers, hydrocarbons possess the same capacity for attaching themselves to cellular proteins[20], and from the result of experiments with labelled hydrocarbons it is hoped to determine the quantitative relationship between this capacity and the carcinogenic potency of hydrocarbons. All these results form a solid basis for Kennaway's conception of chemical carcinogenesis.

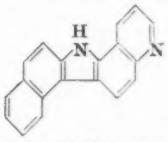
In the course of this work we were led to compare some polycyclic aromatic compounds with various nitrogen analogues. We found that the very large group of heterocyclic compounds analogous in structure to numerous classes of polycyclic hydrocarbons contained an even larger number of substances as active as, or even more active than, the corresponding homocyclic models. For example, the group of angular benzacridines, to which we devoted several years of study[21] includes an even larger number of carcinogens than the related benzanthracenes; a compound such as 3,10-dimethyl-7,8-benzacridine (V) is among the most active substances known.



3,10-Dimethyl-7,8-benzacridine

(V)

More recent work has brought to our notice a fresh class of heterocyclic nitrogenous compounds which promises to be at least as fertile in results as the benzacridine group. This is the azadibenzocarbazole group, some of the members of which are extremely potent, for example, compound (VI).



4′-Aza-1,2 : 5,6-dibenzocarbazole

(VI)

Here, as with the benzacridines, displacement of the heterocyclic nitrogen atom results in a considerable change in biological activity.

One might be tempted to believe that research of this kind, proving as it does that the already considerable number of known chemical carcinogens can be constantly increased, is of purely theoretical interest. In reality, the aim which we had set ourselves from the beginning was very different and very precise: it was to search for a relationship between carcinogenic activity and molecular structure, in the hope of reaching an understanding of the mechanism of chemical carcinogenesis. Earlier authors who had attempted to relate carcinogenic activity to certain molecular structures, such as the position of substituents or the number of rings, had come up against irreconcilable contradictions.

Now, during the Second World War, when France was occupied and only German publications reached us, my attention was attracted by an article by a chemist named Otto Schmidt[22] expounding a theory which appeared to furnish a mathematical explanation of carcinogenesis by polycyclic hydrocarbons, applicable equally to radiation carcinogenesis and even to

virus production! The fact that I could not follow Schmidt's reasoning made me the more curious. I questioned the chemists among my friends; each referred me to another more versed in these matters; until finally I met a young theoretical chemist, Raymond Daudel, who was good enough to take an interest in this work. The author explained the differences in the activity of polycyclic hydrocarbons by calculations of wave mechanics. We were thus concerned with a quantum theory, and since, twenty years earlier, my friend the great physicist Holweck and I had been among the first to explain the biological action of radiations by the quantum theory, I was tempted to try to confirm, by experiment, Otto Schmidt's purely speculative idea.

Dare I say that I had fortunately not seen the latest monograph by Cook and Kennaway, published in 1940 [10]. I should have been discouraged from undertaking any work in this field, had I read their judgment on Schmidt's work: "These conceptions of Schmidt are of interest, but in the absence of exact data concerning electron densities it is difficult to submit them to experimental test. Moreover, like many other theoretical interpretations they are apt to be more successful in explaining known phenomena than in predicting new ones."

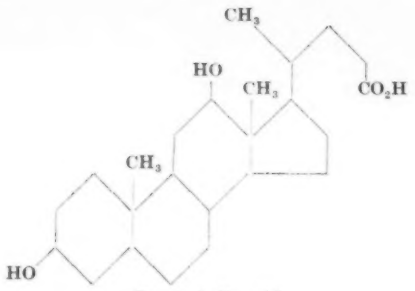
Yet in the twenty years which have elapsed since that time, the rapid progress in theoretical chemistry, aided by the powerful electronic computers, has consolidated the quantum theory of chemical carcinogenesis to the point where it is possible to predict the carcinogenic activity of hydrocarbons before they have been tested. I have neither the time nor the intention to go through the long stages by which the theoretical chemists of the Institut du Radium travelled along this arduous road, thanks to the abundant new material which our organic chemists and experimental biologists had been constantly supplying to the Daudels, the Pullmans and the Chalvets. I may simply recall that it was as a result of the latters' investigations of the electron densities in the molecule of 3,4:9,10-dibenzpyrene (VII), which suggested that this substance might be

3,4 : 9,10-Dibenzpyrene

(VII)

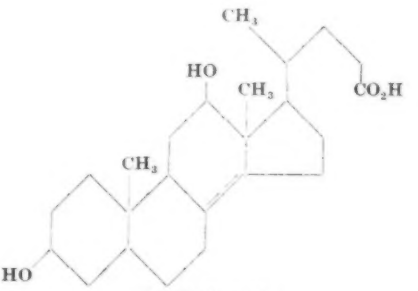
highly carcinogenic, that we undertook its synthesis and test on the mouse; and it proved to be one of the most potent carcinogens known[23]. The close relationship between this biological property and the molecular constitution of the compound indicates that there is a direct causal connexion between them, as Kennaway had assumed.

Again, quite recently, we have confirmed another of Kennaway's important theses and found that the induction of certain spontaneous tumours may be due to certain metabolic variations in the biogenesis or the catabolism of natural steroids. I mentioned earlier the experiment he carried out with Cook and Lady Kennaway[16], which led to the recognition of oncogenic activity in deoxycholic acid (VIII). This result had not been confirmed by other workers, but in collaboration with Buu-Hoï and Zajdela[24] we have just established that another derivative of cholic acid,



Deoxycholic acid

(VIII)



Apocholic acid

(IX)

namely, apocholic acid (IX), is markedly sarcomagenic, and that this result is reproducible at will.

In spite of all this brilliant work, one is obliged to admit that the accumulated research of half a century into the pathogenesis of tumours has still only brought us to the beginning of an understanding of the problem. Nevertheless, one can safely say that the results obtained from the study of chemical carcinogenesis still seem to stand out above all else in this difficult field—even in this era of virus research. They bear witness to Kennaway's worth and to the immortality of his work. His discoveries supplied the scientific bases necessary for the solution of many problems of occupational, preventive and social medicine to which this generous and forward-thinking man dedicated himself during the last years of his life.

I have wondered why I had been chosen to give the first lecture in memory of Sir Ernest Kennaway. Certainly one of the chief reasons must have been the way in which my researches tended in his direction, and the resulting bonds of friendship between the workers at the Chester Beatty Institute and those at the Institut du Radium. But I am inclined to think that account was also taken of the fact that we were contemporaries, he being a few years my senior. We came to maturity during the thirty years preceding the outbreak of the First World War, in a period when the cultivated middle classes, both in Britain and in France, enjoyed complete independence of mind and liberty of action.

It was a period when one could go round the world without a passport, stay at any hotel without filling up a form for the police, profess oneself an agnostic in the political as well as the religious sense without risk of being thought a revolutionary and suspected of a plot to endanger the safety of the State and of society.

We both grew up in an atmosphere where a respect for individual liberty and a veneration for scientific progress were looked on as articles of faith, and we must have been subjected to the same environmental conditions. The two provincial cities where we began our studies were certainly a great distance apart: Exeter, the 'Queen of south-west England', and Lyon, the 'Queen of south-east France'; and yet they are both hard-working industrial towns, with an equally ancient history—*Isca Dumnoniorum and Lugdunum*. In point of fact our scientific careers ran on parallel lines and were devoted purely to impartial research.

I should have failed in my duty if, after paying homage to Kennaway the scholar, servant of human progress, I had not also saluted Kennaway the thinker, who, in philosophy as in science, knew no other guiding star than reason.

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SEASONAL VARIATIONS OF CÆSIUM-137 IN AIR AT GROUND-LEVEL

By K. G. VOHRA, V. S. BHATNAGAR and C. RANGARAJAN

Air Monitoring Section, Atomic Energy Establishment, Trombay, Bombay-73

DURING 1956–58 the levels of radioactive fall-out showed substantial increase in the spring season at several places all over the world. This was attributed to the seasonal effects in the mixing between the stratosphere and the troposphere[1,2]. These conclusions were supported by the observed increase in ozone concentrations in the spring season in the northern hemisphere[3]. An alternative explanation of this spring increase in fall-out was given by Martell[4]. According to him, the spring maximum was observed because of the fact that the U.S.S.R. tests were carried out in the autumn of each year and this gave rise to a maximum of activity on the ground in late winter or spring, a period of 4–6 months being necessary for the matter from fall-out to diffuse from the stratosphere into the troposphere and the ground-level air. However, this explanation was based only on the weapon tests carried out in the polar regions and satisfied the data available at that time.

When the nuclear weapon tests were continuing, it was difficult to decide whether the spring increase was due to the weapons testing schedule or natural seasonal effects, or both. The cessation of nuclear weapon tests after October 1958 provided a period of about 15 months when the measurements could be carried out to study the seasonal variations.

We have been collecting samples of air-borne particulate matter on filter papers since early in 1956. These samples have been collected daily at several stations in India and analysed for gross beta-activity[5]. For finding out whether any seasonal variation existed, we started analysis of these samples for cæsium-137. Since cæsium-137 has a long radioactive half-life, the decay of activity on the filter paper during the period of storage was negligible. The daily samples were pooled for individual months, ashed at a temperature of 400° C., and counted for cæsium-137 using a low-level gamma-ray scintillation spectrometer. The cæsium-137 activity was estimated as monthly average concentration in μμc./m.³ of air.

Fig. 1 shows cæsium-137 concentrations, gross beta-activity, and ratios of cæsium-137 to gross beta-activity at Bombay for the years 1956–60. The dotted lines show the periods for which data were not available. The ratio of cæsium-137 to gross beta-activity gives an indication of the effective age of the

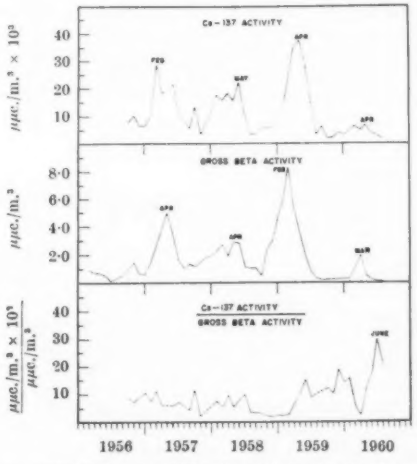

Fig. 1. Activity of cæsium-137 and gross beta-activity of ground-level air and the ratio cæsium-137/gross beta-activity at Bombay

Table 1. Locations of the Sampling Stations

Station	Altitude*	Latitude	Longitude
Srinagar	1,598 m.	34° 06′ N.	74° 55′ E.
Delhi	219 m.	28° 45′ N.	77° 20′ E.
Calcutta	Sea-level	22° 34′ N.	88° 25′ E.
Nagpur	311 m.	21° 12′ N.	79° 04′ E.
Bombay	Sea-level	18° 57′ N.	72° 55′ E.
Bangalore	922 m.	12° 57′ N.	77° 30′ E.
Ootacamund	2,235 m.	11° 23′ N.	76° 40′ E.

* Above mean sea-level.

fission products. Monthly cæsium-137 data for seven other stations in India (Table 1) for the years 1959 and 1960 is shown in Fig. 2, which also includes the levels averaged for all the stations.

Fig. 1 shows that the general trend of variations of the gross beta-activity and cæsium-137 in the air is similar. The peak in gross beta-activity in early 1960 is due to the first French atomic test[6]. The corresponding increase in cæsium-137 due to the French atomic test should be small. Therefore, the increase in cæsium-137 which started in the month of January 1960 and continued until March 1960 should be attributed to seasonal effects. The spring peaks observed in the years 1957, 1958 and 1959 could be due to the combined effects of spring increase and intensive testing of nuclear weapons a few months before the periods showing increase.

The ratio of cæsium-137 to gross beta-activity shows increasing age of the activity after October 1958, when tests were discontinued. However, the ratio shows a sharp decrease during February and March 1960, when the atmosphere contained fission products from the first French atomic test. After March 1960, the ratio increases rapidly, reaching a maximum in the month of June 1960. This also indicates an increase in the concentration of old debris during the months April, May and June 1960.

There are large variations in the levels of cæsium-137 at different stations (Fig. 2), but in general, there is a small increase from late 1959 to early 1960, followed by a decrease. This is more clearly seen in the graph showing the levels averaged for all the stations. This increase is not due to the French atomic test as the contribution of this test to cæsium-137 level in February and March has been estimated to be about 10–15 per cent of the total level from the previous tests. The period of increase at these stations is spread through January to May. These observations may therefore be explained by the seasonal effects in the mixing between the stratosphere and the troposphere as proposed by Stewart[1] on the basis of the Brewer model[7].

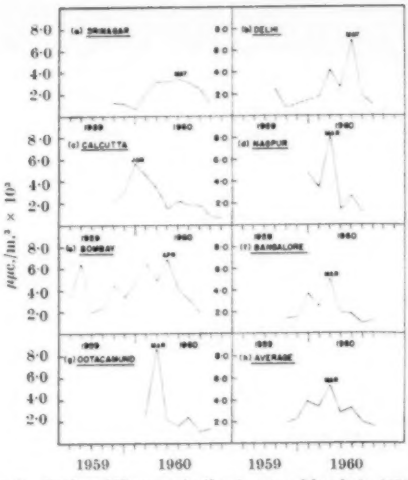

Fig. 2. Cæsium-137 concentration in ground-level air, 1959–60

Recently, there has been evidence from other sources as well on the existence of spring maximum in fall-out[8]. Gustafson has found increased levels of cæsium-137 in surface air during the period April–July 1960, from examination of air filter samples collected at the Argonne National Laboratory[9]. Kuroda, from his work on the concentration of strontium-90 in rainwater[10], and Lockhart, from the measurements of ground-level air-borne radioactivity[11], have also shown the existence of seasonal effects.

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A TAKE-OVER BID IN ACOUSTICAL TRAINING

By Prof. E. J. RICHARDS, O.B.E.
University of Southampton

SINCE noise is essentially related to horse-power, and since this latter commodity is increasing rapidly per man in this industrial community of ours, it was apt that the twelfth scientific conference held by the National Physical Laboratory on June 26–28 in conjunction with the Building Research Station and the National Engineering Laboratory on the "Control of Noise" should concern itself with 'unwanted sound', a topic of great current interest and controversy. In his opening remarks the director, Sir Gordon Sutherland, said that just as "beauty is in the eye of the beholder", then "noise is in the ear of the listener". The enormous width of coverage provided during the three days to the three hundred or so delegates followed as a natural corollary and included a series of papers on acoustical measurements and the various objective and subjective units on the nature of sound sources and methods

suppression, on the ear and hearing conservation, and lastly on community reaction criteria and the possibilities of legislation.

In a way, noise investigations have progressed beyond the stage where large scientific break-throughs are to be expected, and the tone of the conference was therefore one of quiet informativeness rather than belligerent assertiveness. Nevertheless, two items of interest arose time and time again.

The first concerned units, and the need for the establishment of satisfactory criteria of noise-levels which are in keeping with the true subjective reaction of the community. As one speaker lightheartedly put it, "the reason why acousticians are held in disrepute is because they are still arguing emotionally over units. If electrical engineers still regarded electrical current in terms of the nerve stimulus of a dead frog, they equally would be laughed at". Unfortunately, the subjective unit of noise is "emotional" and must include adequately, though maybe not completely, the stimulus of both the ear and the brain. The conference papers did a great deal to pinpoint these basic parameters ; indeed, the papers by D. W. Robinson and Prof. C. W. Kosten were most satisfactory, not so much for their detailed content, as for the impression conveyed that the corner had been turned, and that provided the scientific manpower was available, units acceptable universally to describe aircraft, motor-cycle and factory noises would be put forward during the next ten years.

The second item which needs airing and which was mentioned by many speakers is the provision of trained acoustical engineers. This has up to now been the prerogative of the university physics departments, and it can only be said that both in Great Britain and elsewhere they have taken this duty lightly, even though in fairness it must be said that there are isolated units fighting against the stream of atomic energy and cosmic research at such universities as Durham (Newcastle upon Tyne), London (Imperial College of Science and Technology), Liverpool and Southampton. However, it is significant that since the death of Prof. E. G. Richardson at Newcastle, there has been no single professor of acoustics in Great Britain, and even with the grandiose plans for university expansion there does not appear to be any rush to put right this deficiency. Even at the Imperial College acoustics appears in a lowly place in the expansion process.

Bearing in mind that physicists are being forced to introduce a greater and greater width of fundamental understanding to their students in the three years at their disposal, it may well be that physics departments are justified in this attitude since the basic physics involved in pure acoustics, if not in noise generation, has been established for many years, and the outstanding problems extend into physiology, psychology and engineering as well as into physics. The time has probably arrived therefore when the custodianship of acoustical training should be taken up by the faculties of applied science or engineering and that such a 'take-over bid' should be included in the plans for the next quinquennium. Only in this way can the gross shortage of acoustical and vibration engineers be met.

Such a step is not an unnatural one. Engineering has been defined as the efficient application of the sciences in the interests of the community. Thus a good engineering training, in addition to the teaching of the fundamental sciences and methods of application, also includes some training in economics and in the behaviour of the community. The engineer expects to be interested in 'human engineering', as it is called in the United States, and not only in the purely scientific aspects of his work.

What steps should be taken if the above point of view is accepted ? The establishment at Southampton of a year's postgraduate M.Sc. course in 'vibration and noise studies' to commence in October, and the provision of a readership and lectureship by the Hawker Siddeley Group to support this course is ample evidence of my view. However, a case can also be made out for a 'human engineering' option in the undergraduate stage of the degree in engineering. Such a course could well provide tuition in acoustics, vibration and elementary physiology in the third year in the way that aerodynamics and design, nuclear power and thermodynamics, and hydraulics and soil mechanics is provided now for the aeronautical, nuclear and civil engineer respectively.

Bearing in mind the ever-present practice of putting more and more in a pint pot, the universities themselves favour specialization in the fourth year rather than at the undergraduate stage. However, the pressure from industry for the services of even mediocre students is putting the success of fourth-year M.Sc. courses in jeopardy, and it is to be hoped that industry and the research establishments will themselves see fit to sponsor for such courses students coming into their employ, in order to put right the complaint regarding training so often made at this excellent conference.

OBITUARIES

Dr. Lee de Forest

Dr. Lee de Forest, a pioneer in the history of radio communications and, after much litigation, the acknowledged inventor of the three-electrode thermionic valve, died on June 30 in California at the age of eighty-seven. He was born in August 1873 in the parsonage at Council Bluffs, Iowa, his father being a Congregational minister in that town. Like his father, he graduated from the Yale Sheffield Scientific School in 1896, receiving his Ph.D. degree three years later. While at Yale his interest in electrical phenomena was stimulated by the work of Sir William Crookes and Nikola Tesla and by witnessing demonstrations of Hertz's experiments on electromagnetic waves. In 1899 he went to Chicago to work in the Dynamo Department of the Western Electric Co., and it was there that he became interested in the possibilities of a hot gas or flame detector as an alternative to the coherer then used in wireless receiving circuits.

From this he turned his attention to the Edison effect which he had learnt about at Yale, and which led J. A. Fleming to patent, in 1904, the first diode thermionic valve detector. Two years later, on October 25, 1906, de Forest applied for a patent for a three-electrode valve—a triode—as a device for

amplifying weak alternating currents such as were obtained in the reception of radio signals. This was achieved by applying a voltage to the third electrode inserted between the hot filament or cathode, and the plate, or anode. A few months later, de Forest extended the patent to cover the use of the three-electrode valve as a detector.

With a true inventor's imagination, Lee de Forest foresaw some of the possibilities of radio telephony and broadcasting; and, using an arc transmitter at the Metropolitan Opera House in January 1910, he was the first to broadcast opera arias sung by Caruso and other noted artists.

In 1914 the three-electrode valve was introduced as an oscillator and quickly replaced the arc as a source of continuous oscillations for radiotelephony. It was during the First World War, with the great demands it made on radio communications, that the thermionic valve was rapidly developed and applied to the generation of electromagnetic waves at the sending station, and to the detection and amplification of signal currents at both radio and audio frequencies at the receiver. During the period 1919–24, de Forest devoted most of his attention to the problems of talking cinematograph films, and his phonofilm was an early development in this field.

Altogether he held some 300 patents in the United States and other countries, covering such subjects as the guidance of aircraft by radio beams, the wire transmission of news pictures, and the measurement of cosmic rays. He was awarded the Cross of the French Legion of Honour, and his work was recognized in the United States by the receipt of the Edison Medal and Elliott Cresson Medal of the Franklin Institute, and by Gold Medals at the St. Louis Exhibition in 1904 and the Panama Pacific Exhibition in San Francisco in 1915. He was a founder, Fellow and past-president of the Institute of Radio Engineers (New York), the Gold Medal of which he won in 1922. Apart from various scientific papers, Lee de Forest was the author of *Television Today and Tomorrow* and his autobiography entitled *Father of Radio*.

R. L. Smith-Rose

Prof. B. Hague

Prof. Bernard Hague died on September 29, 1960, in his sixty-eighth year, having occupied the James Watt chair of electrical engineering in the University of Glasgow since 1946, on the retirement of Prof. G. W. O. Howe. Born at Barnsley, Yorkshire, he received his early education at the Grammar School, Eccles, and at the Central School, Rochdale. He started as a millwright apprentice in Rochdale at the age of fifteen and later served as a junior draughtsman with Messrs. Ferranti, Ltd., Hollinwood. He attended evening classes at Rochdale Technical School from where he obtained an exhibition enabling him to study at the City and Guilds College, London. He graduated with first-class honours in 1915, and in the following year obtained the diploma of the Imperial College of Science and Technology. During 1916–20 he was employed as a technical assistant at the Royal Aircraft Establishment, Farnborough, and he began his teaching career with an appointment as a lecturer at the City and Guilds College after being awarded an M.Sc. degree in 1919. His association with the late Prof. G. W. O. Howe, then a lecturer at the City and Guilds College, led to his appointment as a lecturer at the University of Glasgow in 1923, after the appointment of G. W. O. Howe to the James Watt chair of electrical engineering at that University in 1921.

In 1926 he was awarded the Ph.D. of the University of Glasgow, and became a D.Sc. in 1927 and a Fellow of the City and Guilds of London Institute in 1936.

Prof. Hague was a true scholar of wide knowledge and experience. His intellectual pursuits and admirable personal characteristics earned him the respect of many and the loyalty of his staff. He was the author of books on a.c. bridge methods, instrument transformers, electromagnetic problems in electrical engineering, an introduction to vector analysis and the method of symmetrical co-ordination in the theory of polyphase circuits. His *A.C. Bridge Methods* is still regarded as a reference text and has been translated into several languages.

Hague had a particular clarity of presentation both in his writing and in his teaching and this reflected a thoroughness and sincerity of personal endeavour. He appreciated the visual arts and had a deep interest in music, being himself an oboist of distinction. He was a member of the Royal Musical Association and previously a governor of the Royal Scottish Academy of Music.

Above all, his interest in his students was personal and genuine, and he was ever ready to give of himself to the benefit of others, thereby gaining great respect and affection from all with whom he came into contact.

J. Lamb

NEWS and VIEWS

Chemical Defence Experimental Establishment, Porton : Dr. E. A. Perren

Dr. E. A. Perren has just retired from the post of director of the Chemical Defence Experimental Establishment, Porton, after thirty-eight years service in the War Department and Ministry of Supply. He studied chemistry at the Royal College of Science and obtained his Ph.D. in 1922 for researches in Prof. Jocelyn Thorpe's laboratory on three-carbon tautomerism. He then continued to work with Thorpe, as his assistant, at Porton and was absorbed later into the permanent staff there. He was concerned for many years with research into methods of producing smoke-screens, and the results of this work were put to profitable use during the Second World War. His responsibilities afterwards widened and included spells of duty at headquarters and in supervisory work at the Establishment. During 1948–49 he returned to the laboratory to undertake some research in organic chemistry and was then loaned for two years to the Canadian Government to take charge of its Experimental Station at Suffield, Alberta. In 1955 he was appointed director at Porton and retires with the rank of chief scientific officer. Dr. Perren is to continue to serve the Chemical Defence Experimental Establishment in the research laboratories.

Mr. E. E. Haddon

Mr. E. E. Haddon, director of Chemical Defence Research and Development at the War Office, has succeeded Dr. Perren. Mr. Haddon was born at York in 1908. He was educated at Archbishop Holgate's School, York, and at Queen Mary College, London (then East London College), where he graduated with honours in chemistry. After a short period with the Admiralty, he joined the Chemical Warfare Research Department at the War Office in 1929, and during the Second World War became deputy to the Controller of Chemical Defence Development in the Ministry of Supply. In 1945, on promotion to senior principal scientific officer, he temporarily gave up his association with chemical defence to become secretary to the Scientific Advisory Council and assistant director of technical and personnel administration in the Ministry of Supply. He returned to the field of chemical defence in 1952, when he became assistant director, Chemical Defence Research and Development. Five years later he was promoted to the grade of deputy chief scientific officer on becoming director. Mr. Haddon has been closely concerned not only with the direction of chemical defence research in the United Kingdom but also with the framing and implementation of national defence research policy and with the furtherance of international collaboration in this field.

London Transport Directorate of Research : Mr. A. T. Wilford

Mr. A. T. Wilford, director of research, London Transport, is to retire on June 30. Mr. Wilford was educated at Wilson's Grammar School, Camberwell, and obtained his degree at the Imperial College of Science and Technology. During the First World War he served in the Special Brigade, Royal Engineers, and later with the Royal Field Artillery. He entered the service of the former Underground Companies in 1920 as chemist with the London General Omnibus Co. and became chief chemist in 1926, a post which he continued to occupy under the London Passenger Transport Board. He became superintendent of laboratories when the Laboratory Services of the London Transport Executive were centralized in January 1949, and, in May 1950, he was appointed to the new post of director of research. Mr. Wilford has been responsible for the general direction of research activities and scientific control in London Transport and for liaison in these matter with other sections of the British Transport Commission. The new Research Laboratory at Chiswick, which was officially opened in December 1960, was designed in accordance with his requirements. He has served on a number of committees and for several years was a member of Council of the Institute of Petroleum. He is the author of a number of papers concerning the use of fuels and lubricants in road service vehicles and for one of these he was awarded the Crompton–Lanchester Medal of the Institution of Mechanical Engineers.

Mr. H. E. Styles

Mr. H. E. Styles, who is succeeding Mr. Wilford as director of research, London Transport, is fifty-two. He was educated at Strand School, Brixton, and Battersea Polytechnic, where he graduated with first-class honours in chemistry. He joined the staff of the London General Omnibus Co. laboratory, the forerunner of London Transport's present Research Laboratory, in September 1927. In 1940 he became assistant to the chief chemist and, later, assistant to the superintendent of laboratories. Mr. Styles was appointed superintendent of laboratories in 1950, in charge of the Central Laboratory at Chiswick and three laboratories located at London Transport's Lots Road, Greenwich and Neasden electricity generating stations. During the time he has been superintendent, the activities of the Central Laboratory have expanded, both in volume and range, to such an extent as to warrant provision of the newly erected Research Laboratory at Chiswick, in the design of which Mr. Styles was closely concerned. Mr. Styles represents London Transport on the Standardisation Committee of the Institute of Petroleum, the Illumination Industry Standards Committee of the British Standards Institution and the National Illumination Committee of Great Britain. He is chairman of the sub-committee of the National Illumination Committee concerned with Lighting for Transport.

Mr. Isaac Shoenberg : Silver Jubilee of British Television

Following a recommendation of the Television Advisory Committee, an experimental television service began in London in August 1936, with two systems—those of Baird on 240 lines and of Electrical and Musical Industries, Ltd. (E.M.I.), on 405 lines (interlaced). Three months later a public service was formally opened by the Postmaster General; and in February 1937 the Television Advisory Committee recommended that henceforth only the E.M.I. system should be used. In this way the world's first public television service was inaugurated; and what has been sometimes termed the Shoenberg–E.M.I. system has been used by the B.B.C. ever since. Isaac Shoenberg was born in Pinsk, in Russia, in 1880. After taking his degree at the Technological Institute of Kiev, he joined a Russian company and was responsible for research, design and installation of wireless transmitting stations. He came to England in 1914, becoming a British subject five years later. In 1931 he became director of research of the newly formed Electrical and Musical Industries, Ltd. Mr. Shoenberg led the team of scientists and engineers which, in a remarkably short time, developed the 405-line television system, to substantially the same standards as are in use to-day. Mr. Shoenberg was awarded the Faraday Medal of the Institution of Electrical Engineers in 1954 in recognition of his outstanding technical contribution to the British television service. He was the guest of honour at a reception held recently by E.M.I. at Hayes to mark the silver jubilee of the E.M.I. television system.

Status of the Norfolk Broads

In a written answer in the House of Commons on August 1, the Minister of Housing and Local Government, Mr. H. Brooke, pointed out that the primary responsibility for the designation of National Parks in Britain rests with the National Parks Commission. The Commission had advised him that the unique character of the Norfolk Broads made them in no way comparable in type with the other areas which, in conformity with the general requirements of the National Parks and Access to the Countryside Act, had been designated as National Parks. Mr. Brooke concurred in the Commission's conclusion that designation under that Act would not be appropriate, and he said he had agreed that the Minister of Trans-

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port, in consultation with the Minister of Agriculture, Fisheries and Food and himself, will consider the views of the Great Yarmouth Port and Haven Commissioners, the East Suffolk and Norfolk Rivers Board, and other local bodies about the future of the Broads.

International Council of Scientific Unions

The ninth general assembly of the International Council of Scientific Unions will be held at the School of Pharmacy, University of London, during September 25–28. The general assembly will be preceded by meetings of the bureau and of the executive board to be held in the rooms of the Royal Society, which is the British adhering body to the International Council of Scientific Unions. The last general assembly of the Council held in London was in 1946, the first after the Second World War. While the scientific results of the international effort put into the International Geophysical Year are gradually becoming available, plans are being made by the International Council of Scientific Unions and several of the Unions for the "International Year of the Quiet Sun", April 1, 1964–December 31, 1965. The results obtained during the maximum solar activity period of the International Geophysical Year can only be of full value with a complementary programme at the forthcoming period of minimum activity. The disciplines of geomagnetism, aurora and airglow, cosmic rays and space research are particularly affected. Preliminary work is taking place on an "Upper Mantle Project" proposed by the International Union of Geodesy and Geophysics. There is to be a meeting on the subject in Moscow in 1963. The study of the upper mantle and its influence on the Earth's crust is fundamental to the solution of the outstanding questions of geology and solid earth geophysics, and will include work on deep drilling, deep-sea seismographs, deep-focus earthquakes, magnetic and gravimetric investigations, tectonic and magnetic development of crust, work on changes in phase, thermal conditions and equation of state, and high-pressure laboratory work on behaviour of rocks.

The International Union of Biological Sciences has for some time been discussing plans for an "International Biological Programme". Preparation for the programme will take several years, and a preparatory committee of six members has been set up to establish communication lines with the United Nations Organization, national adhering bodies, and other interested groups, and to formulate a precise programme on the basis of information gained. The "Special Committee of Oceanic Research", set up by the International Council of Scientific Unions in 1957, is planning an intensive investigation of the Indian Ocean. A number of reconnaisance cruises has been carried out; but the main period of activity in the Indian Ocean will be 1962–63. With at least thirteen countries and some forty-three ships, the International Indian Ocean Expedition promises to be more ambitious than the oceanographic contribution to the International Geophysical Year.

First Pamphlet on Clean Air

The year 1661 may be said to be the beginning of the campaign for clean air. In that year John Evelyn wrote his famous tract, or discourse, *Fumifugium, or the Smoake of London Dissipated*. The work is dated May 1661, and it was presented to Charles II in September 1661. King Charles, Evelyn records in his diary, "was pleased that I should publish it by his special commands, being much gratified with it". The National Society for Clean Air has now reprinted the tract. It is available either as a paper-back at 2*s*. 6*d*. or cloth-bound at 5*s*. (postage 3*d*. or 6*d*. extra) from Field House, Breams Buildings, London, E.C.4. In the Epistle Dedicatory to "The King's Most Sacred Majesty", Evelyn explains how the idea of the book was conceived: "It was one day, as I was Walking in Your MAJESTIES Palace at White-Hall that a presumptuous Smoake issuing from one or two Tunnels neer Northumberland house, and not far from Scotland Yard, did so invade the Court; that all the Rooms Galleries, and Places about it were filled and infested with it; and that to such a degree, as Men could hardly discern one another for the Clowd, and none could support, without manifest Inconveniency". In his preface to the tract, the author waxes eloquent: "That this Glorious and Antient City, which from Wood might be rendred Brick (like another *Rome*) from Brick made Stone and Marble; which commands the Proud Ocean to the *Indies*, and reaches the farthest *Antipodes* should wrap her stately head in Clowds of Smoake and Sulphur, so full of Stink and Darknesse, I deplore with just Indignation".

Measurement of Quality in the Chemical Factory

In the latest Department of Scientific and Industrial Research booklet which has been produced to extend the application of science in industry, A. J. Clark and C. W. Munday, of the Distillers Co., Ltd., discuss the use of instruments to control processes in the production of chemicals. The authors sum up their attitude with the words, "All chemical plants should have skilled analysts for measuring product quality, and it is indisputable that a first-class analyst is far more versatile than any instrument. However, if it is found that the successful functioning of a process depends on a large number of routine analytical operations, it is worth considering whether an instrument would be a more effective way of doing the job". The advantage of such an arrangement is that the analyst can be released for more creative work, and that continuous control can be exercised without the delay inherent in human supervision. In this booklet, *Quality Measurement in the Chemical Factory*, the emphasis is on instruments of proved worth and relative cheapness, with as wide a range of applications as possible. The authors pay special attention to the use of gas chromatography. Process-stream gas chromatography analysers with a simple repetition time of 1 min. are now becoming available, and, linked to automatic controllers, are already controlling chemical processes. Copies of the booklet may be obtained free from the Department of Scientific and Industrial Research Library, Charles House, 5–11 Regent St., London, S.W.1.

A Soviet View of British Libraries

Under the title "A Soviet View of British Libraries", the Library Association has issued as *Pamphlet* No. 22 a report on a visit in October—November 1959 by V. I. Shunkov, G. G. Firsov and N. I. Tyulina, translated by A. Thompson (Pp. 32, The Library Association, London, 1961. 5*s*.). The pamphlet gives an interesting picture of the learned and public libraries as seen through Russian eyes, of co-operation between these libraries, their staffs, organization, buildings and equipment. Of the learned libraries

only the British Museum Library and the National Libraries of Scotland and Wales are mentioned more than in passing ; of the university libraries only those of Oxford and Cambridge; and the special libraries are even more sketchily noted. There is no reference to any library maintained by a professional society such as the Chemical Society, and the description of the public library service is likewise extremely condensed, though it is obvious from the comments made that the writers of the report have seen and pondered a great deal they have been unable otherwise to mention. The slenderness of most library staffs is stressed, but in noting the favourable comments on buildings and equipment it should be realized that most of the libraries visited were in new buildings, not always completely finished or equipped.

Japanese Planetarium Equipment

Messrs. Chiyoda Kogaku Seiko K.K., manufacturers of Minolta cameras, have now developed a series of Minolta planetarium projectors. These projectors are distributed in the United Kingdom by Japanese Cameras, Ltd., Tunstall, Stoke-on-Trent. There are four standard projector models in the series priced in the range £7,000—£69,000. Models *S* and III are remote controlled and require a dome the diameter of which is in the range 66–100 ft., while models *MS* and *M* are controlled from a position adjacent to the projector and require a dome the diameter of which is in the range 20–50 ft. Models *MS* and *M* project about 3,000 stars, and projectors are installed for the movements of the planets. These planetary projectors are quite separate in the case of model *MS*, but are coupled in the case of model *M*. Model *S* is similar to model *MS* except that it carries a projector giving 9,000 stars and a separate bright star projector. Model III, the most refined of the series, is similar to model *S* with regard to star projection, but incorporates the coupling of the planetary projectors as in model *M* as well as other devices. Each of the four standard projectors carries an equivalent set of special effects projectors, such as a Milky Way projector, a morning- or evening-glow projector, etc. Model III carries a constellation projector in addition. The standard projectors may be supplemented with auxiliary projectors to reproduce auroral phenomena, the solar system as viewed by an external observer, etc. It is interesting to observe that an auxiliary projector can be provided to illustrate the motion of artificial satellites—a choice of American or Russian being provided. It is to be hoped that the advent of cheaper projectors may encourage the establishment of more planetaria in Britain. Small instruments, not necessarily operating on a commercial basis, could be used to assist the teaching of astronomy in schools and colleges, especially if situated in large centres of population.

Medicine, Science and the Law

Three papers, which appear in *Medicine, Science and the Law* (1, No. 4; July 1961), contain implications of considerable importance; each is a real advance in the development and refinement of scientific methods of identifying blood. Dr. R. A. A. Coombs and Barbara Dodd describe a microscopical technique which enables them to determine the nature of a stain on a single fibre of clothing or other material. They show that it is now possible to say with a high degree of certainty not only that the fibre is stained with human blood but also to determine, at least within the limits of the *ABH* system, the group to which the blood belongs. The method is applicable to stains of considerable age, certainly up to three years, in the case of human blood. Dr. H. H. Muller and his colleagues have examined the problem of whether stains consist of human or animal blood in a different way. They have evolved a method of testing for hæmoglobin in the stain. Hæmoglobin differs in its minute physical and chemical characteristics from species to species, but it has hitherto been difficult to identify the species from which it came. The method suggested does not give information about the group of the blood in question. Dr. I. Dunsford, R. Lodge and W. Spielmann describe an investigation into blood groups in a paternity case in which, after a detailed examination of the groups of the child and putative father using all the refinements in technique normally available, the blood groups of the putative father fitted the blood groups of the child in every aspect but one. The child's *Rh* constitution was shown to be *CDE/cde* and the putative father's more probably *CDe/cDE* but possibly *CDE/cde* (that is, as in the child). The technique described in their paper enabled the authors to show that the putative father's *Rh* constitution was *CDE/cde*, so that he was not excluded. Moreover, the 'fit' of his blood with the child's is so good that (although the authors do not claim it) the scientific evidence points to his being in fact the father.

Blindness in Africans in Northern Rhodesia

In 1949 territorial ophthalmic services were started in Northern Rhodesia. Before that date very little was known of the incidence of eye diseases and even less about the extent and causes of blindness. In 1950 a simple blindness register card was produced and distributed to all administrative officers, together with a simple memorandum on eye diseases describing the four conditions which are the principal causes of blindness. Dr. C. M. Phillips, Government ophthalmologist, Lusaka, now shows (*Central African J. of Med.*, **7**, No. 5; May 1961) that the overall incidence of blindness is considered to be 500–750 per 100,000; in some areas it rises as high as 2,300, with the unique feature of having a higher incidence of onset in childhood than in adulthood. The main causal lesions of 2,701 patients examined are corneal pathology, phthisis bulbi, senile cataract and trachoma. It is considered that the predominant factor responsible for the first two lesions is traditional African medicine. Taking all factors into consideration, it is believed that four out of every five persons who have lost their sight did so from a cause that is either preventable or curable.

Uses of Castorseed

A two-day seminar on Castorseed and its Products was held during August 11–12, 1960, at the Regional Research Laboratory, Hyderabad, under the sponsorship of the Council of Scientific and Industrial Research, India. In order to consider the various factors bearing on the present and future of the castor industry in India, a number of questions likely to arise were first formulated under suitable heads. These were sent to a large number of research institutions, organizations dealing with castor, and firms either possessing castor or utilizing its products, especially castor oil. Data or information illustrating and amplifying the questions raised were requested. The data, received in the form of brief notes, were

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arranged suitably under the different heads. These questions and notes were then sent round to all the organizations for thorough study, ensuring that the discussions at the venue would be well informed and fruitful. The entire material has now been brought together in a booklet, *Castorseed and its Products* (Pp. viii+87. Hyderabad: Indian Central Oilseeds Committee. 1961). It includes the questions raised, the notes received and the discussions which later took place at the sessions. Though the seminar dealt primarily with castor in its Indian setting, the material in this booklet might well have a wider interest.

National Council for Technological Awards

The report of the National Council for Technological Awards for the period April 1960 to March 1961 (London: National Council for Technical Awards. Pp. 37) records that nearly 5,000 students were following courses leading to the diploma in technology, compared with 3,800 a year ago, and 309 diplomas were awarded, bringing the total to 472. Approximately 83 per cent of the engineering students and 56 per cent of students of other technologies are working in industry, and the Council is impressed by the increase in the number of students following sandwich courses in technologies other than engineering, from 219 in November 1957 to 1,246 at the end of the year under review; for engineering sandwich courses the corresponding figures are 652 and 3,191. Of 1,769 students enrolled in first-year courses leading to the diploma in the current session, 63 were women. Almost 25 per cent of these students entered their courses by virtue of holding good Ordinary National Certificates, and the Council points out that but for the development of these courses few of these students would have had an opportunity of gaining a qualification equivalent in standard to a university honours degree, and many might never have been added to the nation's technological man-power. The diploma is now accepted as a qualification for entry to the Scientific Civil Service equivalent to a university degree, and most professional institutions exempt holders of the diploma from taking the whole of their own examinations for graduate membership. The Council expresses appreciation of two awards from the Nuffield Foundation in support of study of the nature of courses leading to the diploma: one at the Brunel College of Technology concentrates on the relation of college periods of study to industrial training periods, and the other at the Birmingham College of Advanced Technology is concerned with the spacing of industrial and college training with the object of assembling a body of thought and opinion as a basis for deciding whether changes are needed to improve the quality of industrial and college training, increase the numbers attending advanced courses, and mobilize more effectively the teaching and research resources of colleges and companies. List No. 13 issued also by the Council details 100 existing and 13 proposed courses leading to the diploma.

The Calouste Gulbenkian Foundation, Lisbon

The trustees of the Calouste Gulbenkian Foundation, Lisbon, have announced the grants made during the first half of 1961, which total about £360,000, and, of which, grants in the United Kingdom and British Commonwealth total nearly £100,000. Again they include grants for three years to British undergraduate scientific expeditions, as follows: £200 to the Royal Geographical Society in aid of the expeditions; £200 to the University of Leicester expedition to East Greenland; £200 to the University of Wales expedition to Mount Sabalan; £150 to the Cambridge Arctic Canada expedition; £100 to the University of Oxford expedition to the Andes of Peru; £100 to the University of Oxford expedition to the Atlas Mountains of Southern Morocco; £100 to the Cambridge expedition to Nagir, Karakoram; £100 to the Oxford expedition to the South Caspian; and £50 to the University of Oxford women's expedition to the Madeiras. The grants made in aid of education in Great Britain and the Commonwealth total £41,800, including: £15,000 to the School of Oriental and African Studies, University of London; £4,500 to the Science Masters' Association; £3,900 for three years to the School Library Association. In Portugal, £56,241 has been granted in aid of science and medicine, including grants for the Santa Maria Hospital, Lisbon, for biochemistry at the University of Oporto, the Laboratory of Mass Spectrometry of the Nuclear Energy Study Commission and the Centre of Geological Studies, Lisbon. A total of £7,500 has been given to the Faculty of Engineering of the University of Baghdad. Other countries receiving grants include Brazil (£8,508) and France (£2,612). Grants have also been made to the World Federation for Mental Health and the International Union against Cancer.

Fellowships for Plankton Research

In order to encourage research relating to plankton in the western North Atlantic, the Lamont Geological Observatory is offering several fellowships for independent studies in this field. These fellowships are open to qualified scientists of any nationality, and include transportation costs from home to Sandy Hook, New Jersey, and return, plus a stipend which will be limited ordinarily to 1,500 dollars. Applications and requests for further information should be addressed to the Director, Sandy Hook Marine Laboratory, P.O. Box 428, Highlands, New Jersey.

Carlsberg-Wellcome Travelling Research Fellowships

The Carlsberg Foundation (Copenhagen) and the Wellcome Trust (London) have announced the award of Carlsberg-Wellcome Fellowships for 1960–61 to: Dr. E. Poulsen, senior Lecturer at the Royal Veterinary and Agricultural College, Copenhagen, who will work at the Medical Research Council's Toxicology Research Unit at Carshalton on the neurotoxic influence of alicyclic phosphates and similar compounds; Dr. A. F. Hayward, of the Anatomy Department of the University of Glasgow, who will work at the Carlsberg Laboratory in Copenhagen, under the direction of Dr. H. Holter, studying the electron microscopic appearances in pinocytosis; Dr. S. L. Rowles, a member of the external scientific staff of the Medical Research Council at the School of Dental Surgery in Birmingham, who will work with Dr. Tovborg Jensen of the Royal Veterinary and Agricultural College, Copenhagen, on the chemistry of the calcium phosphates with special reference to those occurring in oral tissues.

Society for Applied Bacteriology

At the annual meeting of the Society for Applied Bacteriology held in Wye on July 11 the following

were elected officers of the Society: *Honorary President*, Mr. G. Sykes; *Honorary Secretary*, Dr. Ella M. Barnes; *Honorary Treasurer*, Mr. G. Elis Jones; *Honorary Editors*, Dr. S. E. Jacobs and Dr. D. J. Jayne-Williams; *Honorary Publications Manager*, Mr. A. H. Walters; *Honorary Advertising Manager*, Mr. E. J. Mann; *Members of Committee*, Mr. B. M. Gibbs, Dr. N. D. Harris, Dr. Agnes A. Nichols, Dr. J. R. Norris, Dr. A. M. Paton, Dr. Patricia M. Scholes, Dr. A. Seaman, Dr. Joan Taylor, Dr. G. C. Ware.

Australian Mammal Society

THE following officers and council members were elected for 1961–62 at the third general meeting of the Australian Mammal Society, held in Brisbane, Australia, on May 26: *President*, Dr. G. B. Sharman; *Vice-President*, Dr. W. D. L. Ride; *Secretary*, Dr. R. G. Chittleborough; *Treasurer*, Mr. J. A. Thomson; *Editor*, Mr. J. H. Calaby; *Council Members*, Dr. A. K. Lee and Dr. A. G. Lyne.

The Night Sky in September

NEW moon occurs on Sept. 10d. 02h. 50m. U.T. and full moon on Sept. 24d. 11h. 34m. The following conjunctions with the Moon take place: Sept. 1d. 23h., Aldebaran 0·3° S.; Sept. 7d. 00h., Venus 2° N.; Sept. 19d. 22h., Saturn 3° S.; Sept. 20h. 05m., Jupiter 3° S.; Sept. 29d. 07h., Aldebaran 0·4° S. In addition to these conjunctions with the Moon, Mars is in conjunction with Spica on Sept. 20d. 11h., Mars being 2° S., Mercury with Spica on Sept. 21d. 12h., Mercury being 0·1° N., Mercury with Mars on Sept. 22d. 21h., Mercury being 3° S., and Venus with Regulus on Sept. 22d. 23h., Venus being 0·4° N. Mercury is too close to the Sun for observation. Venus is a morning star, rising at 1h. 50m., 2h. 30m. and 3h. 10m. on September 1, 15 and 30, respectively. Its stellar magnitude on September 15 is − 3·4; the visible portion of the apparent disk increases during the month from 0·792 to 0·870 and its distance from the Earth increases from 115 to 131 million miles. Mars is too close to the Sun for observation. Jupiter is an evening star, setting at 1h. 30m., 0h. 30m. and 23h. 30m. on Sept. 1, 15 and 30, respectively. It is in Capricornus; its stellar magnitude on Sept. 15 is − 2·2, and its distance from the Earth is 410 million miles. Saturn is an evening star, setting at 1h. 10m., 0h. 10m. and 23h. 10m. at the beginning, middle and end of the month, respectively. It is in Sagittarius; its stellar magnitude is + 0·6 on Sept. 15 and its distance from the Earth is 880 million miles. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Sept. 1d. 23h. 46·7m., σ^2 Tau. (*R*); Sept. 16d. 19h. 54·7m., 24 Sco. (*D*); Sept. 26d. 21h. 25·6m., ξ^2 Cet. (*D*); Sept. 26d. 22h. 19·6m., ξ^2 Cet. (*R*); Sept. 28d. 1h. 40·4m., 5 Tau. (*D*); Sept. 28d. 2h. 46·6m., 5 Tau. (*R*); Sept. 28d. 22h. 50·1m., γ Tau. (*D*); Sept. 28d. 23h. 43·0m., γ Tau. (*R*); Sept. 29d. 3h. 32·9m., θ^1 Tau. (*D*); Sept. 29d. 3h. 45·1m., θ^2 Tau. (*D*); Sept. 29d. 4h. 30·6m., θ^2 Tau. (*R*); Sept. 29d. 4h. 43·2m., θ^1 Tau. (*R*); Sept. 29d. 7h. 57·7m., α Tau. (*D*); Sept. 29d. 8h. 53·8m., α Tau. (*R*). *D* and *R* refer to disappearance and reappearance, respectively. The autumnal equinox occurs on Sept. 23d. 07h.

Announcements

DR. L. R. G. TRELOAR, of the Shirley Institute, Manchester, has been awarded the Colwyn Medal of the Institution of the Rubber Industry. Mr. M. M. Heywood, director of P. D. Cow and Co., Ltd., has been awarded the Institution's Hancock Medal.

A SYMPOSIUM on "Forest Ecosystem" (definitions and classification of forest ecosystem) was held in Montreal in 1959 under the auspices of the Ninth International Botanical Congress. Dr. I. Hustich of Finland was chairman and organizer for the symposium. The papers have now been published in full in a special issue of the *Bulletin of the International Society for Tropical Ecology*. Copies can be had from Dr. G. S. Puri, General Secretary, International Society for Tropical Ecology, 10-Chatham Lines, Allahabad, India (price Rs. 25, £2, 6.00 dollars).

THE Chemical Society is arranging a symposium on electron spin resonance, which will be held in Queen Mary College, London, on November 2. Further information can be obtained from the Secretary, Chemical Society, Burlington House, London, W.1.

THE annual Pittsburgh Diffraction Conference will be held at the Mellon Institute, Pittsburgh, Pennsylvania, during November 1–3. Further information can be obtained from T. B. Massalski, Mellon Institute, 4400 Fifth Avenue, Pittsburgh 13, Pennsylvania.

THE Universities Federation for Animal Welfare (UFAW) will hold its annual Summer Congress at Shipham, Winscombe, Somerset, during September 16–23, 1961. Further information can be obtained from UFAW (Summer Congress), 7a Lamb's Conduit Passage, London, W.C.1.

THE Borough Polytechnic, London, is organizing a course of six lectures on "The Technique of Technical Writing". The lectures will be given on Friday afternoons commencing October 20. Further information can be obtained from the Secretary, Borough Polytechnic, Borough Road, London, S.E.1.

A MEETING of the British Pharmaceutical Conference will be held at Portsmouth during September 18–22. To be included in the programme is a lecture by Dr. A. Isaacs on "Interferon" and a symposium on "Skin Medication". Further information can be obtained from the secretary, British Pharmaceutical Conference, 17 Bloomsbury Square, London, W.C.1.

THE Food Group of the Society of Chemical Industry is organizing a symposium on "Recent Advances in Processing Cereals" at the Royal Society of Medicine, London, during September 14–15. Further information can be obtained from the Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London, S.W.1.

AN international symposium on "Photoelasticity" will be held at the Illinois Institute of Technology, Chicago, Illinois, during October 29–31. The symposium will consist of five sessions including: birefringent coatings; photothermoelasticity and photoplasticity; two- and three-dimensional photoelasticity; dynamic photoelasticity and three-dimensional photoelasticity. Further information can be obtained from Prof. M. M. Frocht, chairman of the International Symposium on Photoelasticity, Department of Mechanics, Illinois Institute of Technology, Technology Center, Chicago, 16.

ERRATUM. In the communication entitled "Effects of Injections of Some Intermediary Metabolites on the 'Insulin Response' of Normal Rabbits", which appeared in the issue of July 29, p. 491, for "trichloroacetic" on line 9 read "tricarboxylic".

THE CARNEGIE UNITED KINGDOM TRUST

THE forty-seventh annual report of the Carnegie United Kingdom Trust covers the year 1960, but also includes a review of the quinquennium 1956–60, in which grants-in-aid of £525,881 brought the total expenditure of the Trust since it was founded in 1913 to more than £6 million*.

Apart from grants which are virtually subscriptions to national bodies and which in 1961–65 will comprise only the National Councils of Social Service in England, Scotland, Wales and Northern Ireland, and Foras Eireann in the Republic of Ireland, the Trust's grants fall into two main categories: those used to finance special inquiries and experiments, and those which provide a wide range of equipment and services. The first category gives the Trust scope to support innovation and pioneering in the arts, in social services and in education, but during the past quinquennium the largest proportion of grant-aid has gone to beneficiaries in the second category, particularly for the re-equipment of village halls and for museums. During the quinquennium, grants for the arts accounted for 11·72 per cent, for community services for 56·58 per cent, physical and mental welfare for 6·99 per cent, education for 15·5 per cent, youth services for 0·62 per cent, and nature reserves and parks for 8·59 per cent, the last including a grant for the David Marshall Lodge, Aberfoyle, opened in 1960, in the Queen Elizabeth Forest Park, and a sum of £50,000 has been allocated for the provision of other suitable amenities in such special areas during the 1961–65 quinquennium, out of a total allocation of £416,500. Other allocations for 1961–65 include £45,000 for museums, £100,000 for new communities, £100,000 for youth service and £10,000 for field studies, which will cover grants for projects approved and recommended by the Council for British Archæology and by the Council for Nature, both for field work in natural history and for Conservation Corps projects.

During 1960 the Trust continued its policy of assisting the development of museums and their associated art galleries, and 16 museums were offered grants for modernizing their displays; but the Trust does not consider that the museum service generally receives the support it deserves, though it is watching with interest certain developments which promise to deal with the situation. During the quinquennium, grants amounting to £49,930 brought the total aid from the Trust during 30 years to nearly £150,000, not including £16,695 promised to 38 museums towards expenditure on reorganizing displayed collections and a special grant of £1,000 to the Royal Burgh of Culross for work on an attractive old building, "The Study", which the National Trust for Scotland has restored for use partly for residential purposes, partly as a small museum and partly as an information centre, and another of £10,000 towards the capital cost of a Shetland Museum and Art Gallery at Lerwick. For the next quinquennium, the Trust's grants for museums will continue to be devoted largely to encouraging modernization of displays.

Although the last quinquennial report recorded the completion of the Trust's policy of grant-aid for the building or adaptation of village halls in the United Kingdom, an inquiry in 1956 convinced the Trustees that there were needs not being met from public funds, and they decided to return to this field with an allocation of £100,000 for grants for refurnishing and re-equipping pre-war halls in villages of not more than 1,000 people. By the end of 1960 some 1,636 applications had been received and the allocation increased to £200,000. During 1960 promises numbering 216 brought the total to 1,267 and the commitment to £171,031. Two more schemes for film projectors in the Highlands were approved on the recommendation of the Highlands and Islands Film Guild and a set of equipment is being presented to the people living on the Skerries, off the coast of Shetland. The social problems of new communities have received special attention during the quinquennium, and a report by Dr. J. H. Nicholson on his study of four types of new community is being published this year under the title "New Communities in Britain: Achievements and Problems". The £100,000 allocated for grants in this field will be available for projects that are truly pioneer, and likely to serve as prototypes, if successful; a grant of £12,000 has already been promised for the building of the Family Welfare Centre of the Kirkby Council of Social Service, and a tapering grant of £3,000 over 5 years to Hartcliffe Community House, Bristol, for provision of a warden and a qualified social worker.

A grant of £1,000 was made to Gates Youth Club, Crawley, to assist in building a permanent youth club and one of £8,000 to Edinburgh University Settlement for the erection of a multi-purpose building to serve primarily as a youth centre and also as a training ground for youth leaders. The Trust has also promised half the total cost of £13,000 required to establish the Northern Ireland Association for Mental Health for its first five years, and a supplementary grant of £3,500 was offered to the National Association for the Paralysed, for connecting to mains drainage several buildings at Dorincourt for work among the young chronic sick. £5,000 on a £1 for £1 basis after the first £10,000 was assured was offered to the Edinburgh Cripple Aid Society for a scheme to cost £20,000 for establishing an up-to-date centre for all physically handicapped persons who require assistance, and a grant of £2,250 was promised to the Y.W.C.A. for the salary and expenses of a part-time research assistant, to work for three years under supervision from the head of the Social Science Department, London School of Economics, for an experiment in social work among adolescents based on a coffee bar in the Paddington area of London.

During 1960, 120 students from 75 local scientific societies received bursaries to attend residential courses in botany, zoology, geology, ecology, archæology and related subjects at a cost of £360, and 17 grants, totalling £1,091, were made for field work in archæology. Nine courses for instruction of

* The Carnegie United Kingdom Trust. 47th Annual Report, 1960. Pp. viii + 98. (Dunfermline, Fife: The Carnegie United Kingdom Trust, 1961.)

amateurs in meteorology were arranged, five of which were vacation courses at the Field Study Centres of Malham Tarn, Preston Montfort and Dale Fort, and three week-end courses at Lasham, Longmynd and Dunstable. Grants for youth service in the new quinquennium will be substantially for refurbishing and improving existing youth clubs. A grant of £12,750 payable over 5 years from the beginning of 1960 has been promised to the Central Council for the Care of Cripples to establish and develop the Homecrafts Advisory Association for the Disabled.

THE REGIONAL RESEARCH LABORATORY, HYDERABAD

THE annual report for 1959–60* of the Regional Research Laboratory, Hyderabad, is a substantial attractively produced volume printed on hand-made paper manufactured by the Laboratory. It includes lists of research staff, auxiliary technical staff and administrators' staff and of publications and patents, and gives fairly full details of research projects in progress. Most of the projects relate to some industrial process or product, but increasing emphasis is laid on the fundamental aspects of applied problems. A Specialists' Committee, under the chairmanship of Prof. M. S. Thacker, has been appointed to examine the research programmes and make recommendations broadly on the future scope of the research programmes undertaken by the Laboratory.

The work of the Laboratory continues to range over a wide field. In oils and fats, the keeping quality of castor oil and cottonseed oil claimed much attention, as well as the preparation of derivatives. A series of programmes on surface coatings dealt with dehydrated castor oil, its isomerization and styrenation, and the preparation of varnishes from dehydrated castor oil, as well as the preparation of alkyds, emulsion paints, the weathering of paints and the use of cashewnut shell liquid. Syntheses in the quinoline, isoquinoline and thieno-pyrimidine series were directed to the preparation of compounds of potential pharmacological interest, while a process for the manufacture of phenylacetic acid was re-examined, and 8-hydroxyquinoline and 5-chloro-7-iodo-8-hydroxyquinoline synthesized for treatment of amœbic dysentery. Schemes relating to essential oils and aromatic chemicals deal with palmarosa oil, Indian cinnamon leaf oil and the utilization of Indian turpentine. In addition to entomological work on insecticides, investigations have been carried out on the effect of X-rays on the biological and biochemical characteristics of the spermatozoa of insects.

In biochemistry, the microbiological production of calcium and ferrous gluconate and of citric acid, the effect of cell concentration on the biological properties of individual cells in cell suspensions, and the biosynthesis and metabolism of proteins and nucleic acids have been examined. Programmes dealing with fuel cover the low-temperature carbonization of non-caking Indian coals and the utilization of products of the low-temperature carbonization of coal. Activated carbons and bleaching earths continue to receive attention, and there are programmes dealing with titanium pigments, the manufacture of white cement from felspar and the preparation of catalysts for the low-pressure hydrogenation of tar. Physico-chemical investigations dealt with the mineralogical composition of the bleaching earths, spectro-photometric work on tar acids from low-temperature carbonization, the surface properties and heat of wetting of absorbents and catalysts.

Under chemical engineering is included work on the vapour–liquid equilibrium of fatty acids and tar acids; the fluidized activation of carbon; pilot plant production of lævulinic acid and lounginin (a synthetic flavouring agent); and pilot-plant investigations in the milling of cotton seed and castor seed.

* Regional Research Laboratory, Hyderabad. Annual Report, 1959–60. Pp. xiv + 217. (Hyderabad: Regional Research Laboratory, 1961.)

BIORHEOLOGY AND MICROCIRCULATION

FOLLOWING a reception at the Hadassah Visitors' Club, a seminar on biorheology and microcirculation was opened on April 24 at the Medical School, Jerusalem, by Dr. K. J. Mann, the general director of Hadassah, and delegates were also welcomed by Prof. M. Rachmilewitz, dean of the medical school, Hebrew University.

Dr. E. Davis (Jerusalem) introduced the symposium with a paper on the general nature and problems of microcirculation as studied by optical and electron microscopy. A main problem is how the capillaries change in diameter—whether passively in response to changes in blood content, or actively in response to nervous or chemical stimuli. How red cells pass through the apparently intact capillary is also still a mystery, nor are the spontaneous changes in calibre and rate of flow of blood in the small vessels understood.

Introducing the biorheological aspect, Dr. G. W. Scott Blair (Reading, England) outlined briefly the history of the application of rheology, "the study of the deformation and flow of matter", to biological systems. Poiseuille, a pioneer of rheology, and Bingham, who named this branch of physics and organized the first society of rheologists, both worked on problems of blood-flow. Biorheologists now study many other systems, such as muscle, protoplasm, cervical mucus, synovial fluids, bronchial mucus, intraocular and cerebrospinal fluids, strength of bones, etc.

Dr. H. Harders (Hamburg, Germany) stressed the technical difficulties involved in direct microscopic observations of microcirculation *in vivo*. But the

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importance of such studies is undisputed. Many diseases have microcirculatory aspects, and some, such as sickle-cell disease, cold hæmagglutination syndrome, polycythæmia and hæmorrhagic thrombocythæmia, are primarily disturbances of microcirculation. The influence of vaso-active drugs and of smoking can be studied by microscopic examination of capillaries. Dr. Harders concluded by predicting that microvascular observations would, in future, play an essential part in routine examinations.

Dr. J. W. Irwin (Boston, Massachusetts) showed, with a film, the pulmonary microcirculation in the living rabbit. The arterioles, capillaries and venules could be seen, and the pulsating flow was readily observed. Pharmacological reactions were also demonstrated. A capacitance electromanometer was shown which could be used, provided that the tip of the microcannula was less than 20μ in diameter, but a more efficient manometer would be needed if true capillary pressures were to be studied.

Dr. J. Weinman and Dr. M. Manoach (Jerusalem) described photoelectric techniques for studying the peripheral circulation in man. Living tissue is transparent to those wave-lengths of red and infrared radiation at which measurements of extinction coefficients of blood are generally made. Quite cheap incandescent lamps and photoconducting cells may be used as radiation sources and detectors to follow pulsating flow of blood and vasomotor action. Photocells and light sources are combined in a small unit, attachable to any part of the body. Such stimuli as postural gravitational changes and smoking may be studied in this way.

The last session at Jerusalem was opened by Prof. A. Katchalsky (Rehovot), who described differences in osmotic fragility under normal and gradual conditions of hæmolysis. Under normal hæmolysis, stresses develop rapidly and rupture the cell membrane. Under slow hæmolysis a higher hypotonicity is required. The membrane behaves as a Kelvin body with a characteristic retardation time. Dr. D. Danon (Rehovot) showed two phase-contrast microcine films illustrating deformation, shearing and hæmolysis of normal human erythrocytes under gradually decreasing salt concentration. Mixed ion exchangers and also dialysis through a 'Cellophane' membrane were used to reduce salt concentrations. Dr. G. Segré and Dr. A. Silberberg (Rehovot) studied the radial distribution of rigid macroscopic spheres in a Poiseuille field of flow, showing that particles undergo a sideways displacement, both from the centre outwards and from the tube wall inwards. Although these results should be applied with caution to flow of blood, M. G. Taylor, scanning a capillary optically and measuring absorption along a diameter, has shown a similar 'tubular pinch' effect.

The second part of the programme, held at the Weizmann Institute of Science, Rehovot, opened with a paper by Dr. S. Lazarus, Dr. H. N. Munro and Dr. G. H. Bell (Glasgow) on capillary strength tests in scurvy and their reactions to vitamins C and P therapy. Both positive and negative pressure tests were used on three small groups of subjects of about the same age: (1) fifteen cases of scurvy; (2) twenty-nine hospital in-patients without scurvy; and (3) twenty well-nourished hospital visitors. Negative pressure tests were inconcuslive, but positive pressure tests showed some weakness in the capillaries of scurvy patients, though more than a quarter of them fell within the normal range of group (3) subjects. Treatment with vitamins C and P did not increase capillary strength. The authors tentatively suggested that it was not scurvy which produces capillary weakness, but that subjects with already weak capillaries are perhaps more prone to develop scurvy.

Dr. S. Ben Hador, Dr. E. Davis, Dr. J. Landau and Dr. M. Ivri (Jerusalem) discussed the effect of adrenaline on the small blood vessels of the conjunctiva and nailfold, concluding that these vessels are more sensitive to adrenaline in the presence of hypertension than when blood pressure is normal. As an example of the results presented: injecting 0·4 mgm. adrenaline produced severe thinning in the small vessels of the nailfold in two of seven healthy subjects, and appreciable thinning in two more cases; whereas, among eleven hypertensives, severe thinning appeared in six, and appreciable thinning in five, cases. Among ten arteriosclerotics, there was no severe thinning and there were six cases of appreciable thinning.

Dr. J. W. Irwin (Boston, Massachusetts) discussed microcirculation of the inner ear, and showed a film demonstrating this in living guinea pigs. A remarkable and unexplained finding is that the chemical components of endolymph and perilymph, which the author considered as part of the circulation of the inner ear, appear to differ, although these fluids are separated in one area only by a two-cell thick membrane (Reissner's membrane).

Dr. E. Davis, Dr. S. Ben Hador, Dr. J. Landau and Dr. M. Ivri (Jerusalem) then described observations and experiments on vasomotion in human capillaries. The number of spontaneous changes a minute in calibre or velocity of blood-flow in capillaries is defined as the 'vasomotor count'. In healthy subjects and in arteriosclerotics, the vasomotor count lies normally between 0 and 14, but in hypertension and in diabetes mellitus, two-thirds of the cases showed values greater than 14. Injections of 0·4 mgm. adrenaline showed no change in vasomotor count in healthy subjects, but increases in 7 out of 11 cases of hypertension and also 3 out of 9 arteriosclerotics. The increased vasomotion of hypertension may reflect increased sensitivity to adrenaline.

In the final paper, Dr. G. W. Scott Blair (Reading, England) briefly outlined the history of rheological theory, from Hooke's law of elasticity (stored energy) and Newton's concept of viscous flow (dissipated energy) to modern theories of entropy-high elasticity. Living organisms maintain themselves by preventing fatal rises in entropy through drawing 'negative entropy' from their surroundings. In a steady, as distinct from a static, state a system undergoing an irreversible process requires a rate of increase of entropy having the minimum possible value consistent with external conditions. Dr. Scott Blair predicted that it is along the lines of Onsager and Prigogine's 'irreversible thermodynamics' that rheological theory must develop if it is to deal with studies of living systems.

Prof. M. Reiner (Haifa), who presided at one of the sessions, discussed the function of models in biorheology and, in a final general discussion, views were expressed concerning the future international organization of biorheology, especially the part which might be played by the new international journal *Biorheology*, shortly to appear.

Apart from the technical sessions, there were many pleasant social activities at which the traditional Israeli hospitality was seen to its full advantage.

G. W. Scott Blair

PAPER CHROMATOGRAPHY

THE technique of paper chromatography was first described by Consden, Gordon and Martin in 1944. In 1948, Dr. Gordon was invited to Czechoslovakia for a year to set up and teach this technique. Since that time the work of the Czech Schools has become more and more important in this field and there can be little doubt that, at present, the work of Drs. I. M. Hais, K. Macek, C. Michalec and their co-workers is among the most important of the systematic and general research in paper chromatography. So far as we are aware, the Czech Chemical Society is the only chemical society which has a Chromatography Group, and it is therefore not surprising that it should have organized a conference on the subject of paper chromatography during July 21–24, 1961.

The conference, using Czech, English and German as official languages, was organized so that the first two days were given to the discussion of new techniques, reviews of particular fields, papers relating chromatographic behaviour to chemical and physical properties and to the paper chromatography of lipids—a subject to which the Czechs have devoted a very great deal of time—and the third day to a discussion of theoretical and practical problems. As many papers were read in Czech, books containing adequate summaries in various languages were handed to each participant in advance and interpreters were assigned to individual national groups. The first two days were considered as open days, and students and others interested were encouraged to attend. The third day was arranged as a closed meeting confined to about sixty participants, which was held in a hall with radio equipment and transistorized headphones on which were relayed simultaneous translations in English, German and Russian. For this the participants were transported to a small castle outside Prague which the Government has made available to the National Academy of Sciences as a holiday resort for scientists.

In the introductory review, Dr. R. Consden discussed briefly the origin and development of paper chromatography and went on to a consideration of the various new papers, now becoming available, and new techniques, such as centrifugal field methods. Other speakers then reviewed particular subjects. Thus Dr. C. Knight of Balston, Ltd. (Whatman filter papers), spoke on the new ion exchange papers in which the active exchange group is built into the cellulose molecule. He described the cation exchangers such as cellulose–phosphate and citrate and carboxymethylcellulose, together with their application to the separation of amino-acids and cations in general and followed with a similar discussion on the cellulosic anion exchangers including diethylaminoethyl cellulose. Similar papers were also discussed by Dr. Grüne, of Schleicher and Schüll, who went on to consider the value of new types of paper which have alumina or silica gel incorporated into the cellulose matrix at the manufacturing stage. She pointed out that these papers function as micro alumina or silica gel columns and yield results similar to the classical column procedures. They are thus useful for the examination of new solvents for column chromatography as well as for separations in their own right. Among many examples quoted we may mention only the separation of carotenoids and chlorophylls on these papers.

There followed reviews on the separation of particular families of compounds and a few may be taken for special comment. Dr. F. H. Pollard gave an interesting review of present work and advances in the inorganic field. In particular, the search for new general and specific location reagents and for faster solvents had been quite successful. In a second paper he described a method for the rapid systematic analysis of some twenty-four cations using a single solvent in conjunction with a large number of reagents for location and confirmation of identity. This method had been used most successfully by students in place of the classical inorganic group separations. Results of Australian workers in the inorganic field were discussed by Dr. M. Lederer. An excellent review of the chromatographic separation of some two hundred alkaloids on formamide-treated paper was given by Dr. D. Waldi (Germany), who directed attention to the possible errors due to variation in the formamide itself.

One whole session of the conference was devoted to the paper chromatography of fatty acids, esters and related compounds. Following a comprehensive review by Dr. Michalec on solvents, reagents and techniques useful in this field, other papers discussed particular series of compounds. Methods were described for the separation of *cis–trans*-isomers of the acids together with the application of this work in the determination of the mechanics of catalytic hydrogenation. Other speakers dealt with the use of coated papers, such as formamide or dimethylformamide-treated papers, for the separation of free fatty acids or their urea adducts, and the quantitative methods available for esters, glycerides, etc.

The only paper devoted to the theory of paper chromatography was that of Prof. V. V. Racinskij (U.S.S.R.), who discussed chromatographic separation in terms of the general theory of sorption dynamics. Like other theories, this theory requires the measurement of the cross-sectional areas of both phases on the paper. However, as it has not yet been found possible to determine these areas, we must agree with Prof. Racinskij himself, who concluded by directing attention to the lack of applicability of these calculations and theories to everyday paper chromatography.

Two films were shown near the beginning of the meeting. One entitled "Principles of Chromatography", by Drs. J. B. Jepson and I. Smith, illustrated the general procedures and techniques of paper and column chromatography, while the second, by Drs. V. Holeysovsky, V. Tomasek, K. Kochloefl and K. Setinek, was a complete description of the "Technique of Paper Chromatography of Amino-acids" as carried out in Prague. Taken together they formed a suitable introduction to the conference for the many students present. While the British film is available on loan or sale it is regrettable that the Czech film is not.

The original intention for the closed meeting on the third day was to have a symposium on the relationships between chemical structure and chromatographic behaviour in the morning followed by a symposium on systematic analysis in the afternoon. In a sense, the whole three days were devoted to these topics;

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but, despite the very long hours put in each day, there was insufficient time for adequate and fruitful discussions.

The main discussion at the two symposia, at which Prof. Marini-Bettolo (Italy) presided, centred around the papers of Drs. Hais and Macek, and included contributions by Drs. Consden, Smith, Waldi, Racinskij and others. They emphasized the fact that the vast amount of chromatographic data in the literature was not being used to the full simply because of the lack of standardization of apparatus, methods, reagents, etc., and that this rendered comparisons and deductions between the results of different workers dangerous. They directed attention to the very wide and detailed systematic studies of Dr. I. Smith in the biochemical field and to the limited number of such studies in other fields. We were interested to learn that the Soviet Union does not produce a single piece of apparatus which is primarily intended for paper chromatography. The variety of types of apparatus used by workers in different countries is unfortunately manifold. Because of these differences it is not yet possible to correlate the data produced in different laboratories on anything like the scale which would be desirable. This is regrettable, as there can be no doubt that standardized and correlated data would be valuable in avoiding the present duplication and reduplication of labour. Moreover, far more important than this, standardized data could be used on electronic data processing machines based on suitable punched-card systems. Furthermore, the availability of such standardized data would be valuable for the determination of various other chromatographic data, such as R_m values, and for the correlation of such properties with other physicochemical measurements. This in turn might lead to a type of structural analysis based on the splitting of R_F and R_m values into functional group constants with the possible application of physical-organic chemistry to the general problems of paper chromatography. This latter point was discussed at some length by Ing. Prochazka and Ing. Gasparic. The Czech Chemical Society has attempted with some success to standardize methods in its own country and, in spite of difficulties, Drs. Macek and Hais have gone ahead and devised their own punched-card system suitable for machine operation. No doubt we can look forward to more interesting developments from their laboratories.

Participants were shown around two laboratories. Dr. Macek conducted a tour of the Pharmaceutical and Biochemical Research Institute, which is well equipped to study all aspects of paper chromatography, including the data-correlation problem. This was followed by a tour of the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Science, led by Prof. B. Keil. The main problem of Dr. Keil and his team is the amino-acid sequence of chymotrypsinogen and the laboratory is extremely well equipped for this purpose. The electronic data sorting approach to the study of overlapping sequences, etc., is also in an advanced stage. We are interested to note the close collaboration between this team and others in the United States working on the same problems.

I. Smith
J. B. Weiss

FOREST PRODUCTS RESEARCH IN BRITAIN

THE extreme complexity of the principal product of the forest, timber, is well exemplified by the very varied contents of the report for 1960 of the Director of Forest Products Research*. In it reference is made to research into wood structure, wood seasoning, bending, mechanics and other properties of timber. Sections are devoted to woodworking, wood preservation and to the study of the fungi and insects which batten on timber. The chemistry and physics of timber are receiving attention and in this connexion surely more attention should be given to the chemistry of those timbers of trees which from a sylvicultural point of view ought to be much more widely grown, especially in England, than they are—namely, oak, birch, alder and other leaf-trees. It would be very helpful if timbers containing substances which are a nuisance in paper-making, but which might furnish materials for other uses, could be enhanced in value and grown more profitably. Recent progress in Japan is of interest.

The close association of the Forest Products Research Laboratory with the Forestry Commission no doubt accounts for the prominence given in the research programme to Sitka spruce and what is loosely called "Lodgepole pine". The former has been very extensively planted and the latter, after a period of neglect, is now also much in favour. Both are useful trees, and it is certainly important that everything should be done to justify their being grown, but surely not at the expense of work on other sylviculturally more useful trees with no less valuable timber. The Republic of Ireland, where the utility of *Pinus contorta* Doug. was appreciated earlier than in Great Britain, is indeed fortunate in being able to make use of the Laboratory, while the latter is fortunate in being able to obtain research material from abroad suited to its needs.

An investigation just completed into the strength and specific gravity of Sitka spruce grown in Great Britain proves fairly conclusively that the method of site classification adopted by the Forestry Commission is at fault. The samples tested were chosen from six geographical regions corresponding to 'Districts' of the Meteorological Office, in other words, climatic regions, and within these the stands of trees, whence the samples came, were classified into five groups based on rate of growth in height, called, unfortunately, quality-classes, but really productivity-classes. The interesting result of this investigation was to find no significant differences among the six climatic regions, nor among the productivity-classes as a whole, but that differences in quality tend to arise more from individual site factors. It thus seems that rate of growth is not a sound basis of site classification for this kind of work, as most foresters realize. A better classification is one based on the factors of geology and the availability to the trees of nutrients and soil moisture. It would be valuable to have the work repeated or the existing data re-analysed on such a basis.

* Department of Scientific and Industrial Research. *Forest Products Research 1960*: Report of the Products Research Steering Committee with the Report of the Director of Forest Products Research. Pp. v + 60 + 8 plates. (London: H.M.S.O., 1961.) 5*s*. net.

The report does not say specifically what the value of the timber of *Pinus contorta* Doug. is compared with that of other trees, information which would be of the utmost importance to the forester. The structure of its wood is said to be intermediate between, for example, yellow pine and Scots pine. There is some slackness in nomenclature in the report; apart from leaving one in doubt about what yellow pine is, the terms 'redwood' and 'whitewood' are still used with no explanation that the former is wood of Scots pine and the latter that of Norway spruce. The laboratory should think of the grower as well as of the user.

It is almost certain that timbers will long remain among the most useful of raw materials, and the more we can learn about them the better. As the chairman of the Forest Products Research Steering Committee in his introduction says, there is no lack of work to be done, but why is all the work kept in the south of Britain? Some could be done just as well at a properly equipped laboratory in the north. The Committee has a difficult task in avoiding the many reefs and in selecting the most profitable waters. The greater attention now being given to home- (and Irish-) grown timbers is, however, likely to be fully justified.

M. L. ANDERSON

DRUG SUPPLIES DURING THE AMERICAN REVOLUTION

INITIALLY, drug supplies for the American Revolutionary Army came from stocks largely in the hands of private druggists. This source of supply was totally inadequate for a war that attained such proportions as the Revolution. Even if stocks of drugs in the Colonies had been far greater than they were, there is little reason for believing that shortages would not have developed. Even the most patriotic pharmacists were faced with complete financial suicide, caught between a spiralling inflation and a Congress that had no money and only a promise for the future. In an interesting paper, George B. Griffenhagen, formerly curator of medical sciences, U.S. National Museum, Smithsonian Institution (*Bull.* 225), shows how the internal organization of the medical department of the army was so chaotic that, even if adequate supplies were available and if the almost unsurmountable problems of communications and transportation had been solved, it is almost certain that shortages would have developed at least during the campaign of 1776.

The encouragement which Congress, through its Secret Committee, gave to private shippers for the importation of vital war supplies offered little relief in the field of medical supplies. Importation was cut off from Great Britain, and France did not directly export any quantities of medical supplies, at least until 1778. American privateers found it much more profitable to prey on British shipping than to develop trade channels with countries which, prior to the Revolution, were prohibited from shipping directly to the Colonies.

Hence, the most immediate relief from medical supply shortages was provided by the American privateers. Drug cargoes from British prize ships, many of which were en route to New York, served as a most important source of supply, particularly during 1777 and 1778.

The number of individual drugs mentioned in various inventories was considerable. Of these, only about a dozen were critically short. Heading the list of these 'capital articles' was Peruvian bark, the same cinchona from which quinine was later discovered. Tons of bark were used during the Revolutionary War, and the price more than quadrupled between June 1776 and September 1777.

The most prominent group of drugs on the list of capital articles consisted of cathartics and purgatives. Jalap, ipecac, and rhubarb were the botanical favourites, while bitter purging salt (Epsom salts) and Glauber's purging salts were the chemical choices for purging. Tartar emetic was the choice for a vomit, and cantharides (Spanish flies) was the most important ingredient for blistering plasters. Opium was administered for its narcotic effects, while gum camphor, nitre (saltpetre or potassium nitrate), and mercury (pure metal as well as certain salts) were employed for a variety of purposes. Lint, a form of absorbent material made by scraping or picking apart old woven material, was also in short supply.

Equipment shortages included surgical instruments and mortar, and pestles for pulverizing crude drugs. Glass vials for holding compounded medicines were also a supply problem, especially after essential drugs again became available. Some of the shortages were eased by local manufacture. Lint was produced in large quantities in the Colonies, and glass vials were manufactured in numerous glasshouses. Local manufacture of purging salts and nitre aided in eliminating shortages of these essential items, and, at the same time led to the first large-scale pharmaceutical manufacturing in the United States.

π-ELECTRONIC EXCITATION AND IONIZATION ENERGIES OF CONDENSED RING AROMATIC HYDROCARBONS

By Dr. J. B. BIRKS and M. A. SLIFKIN
Physical Laboratories, University of Manchester

THE condensed ring aromatic hydrocarbons form an interesting group of molecules. They possess delocalized π-electron systems, loosely bound by a potential defined by the shape of the planar molecular skeleton; they scintillate, fluoresce and phosphoresce; some of the higher members are carcinogenic; and they possess certain well-defined electronic energy-levels which vary systematically with molecular size. They have been the subject of many theoretical studies, including those based on the molecular

orbital[1-3], valence-bond, and free-electron[4] models. These have led to the classification and computation of the π-electronic energy-levels in many of the simpler compounds, although because of the complexity of the many-electron systems and the resultant interactions it is normally necessary to introduce arbitrary parameters to achieve reasonable agreement with the experimental data.

The electronic ground-state S_0 of the molecules is singlet, and analysis of the visible and ultra-violet absorption spectra ($S_0 \rightarrow S_n$) of vapours or dilute solutions yields direct information on the singlet π-excitation energy-levels S_n. Complementary data on the energy and life-time of the first singlet excitation state S_1 are obtained from studies of the molecular fluorescence emission, $S_1 \rightarrow S_0$.

The triplet π-excitation energy-levels are less amenable experimentally, since singlet-triplet absorption transitions are normally forbidden. The first triplet π-excitation energy T_1 can be obtained from observations of the low-temperature phosphorescence emission ($T_1 \rightarrow S_0$) of glassy solutions[5], or by the solvent perturbation technique[6] which allows the $S_0 \rightarrow T_1$ transition to be observed in absorption at room temperature. Higher triplet states have been observed in transient $T_1 \rightarrow T_n$ absorption transitions, using the flash photolysis method[7].

The ionization energies of a few of the smaller molecules and their derivatives have been obtained directly from photo-ionization measurements[8], electron impact studies[9] and vacuum ultra-violet Rydberg spectra[10] of the vapours. These methods are not applicable to the heavier, less volatile compounds, and an indirect technique[11,12], involving the measurement of the absorption spectra of the charge-transfer complexes formed by the compounds with an electron acceptor in solution, has been used to obtain the ionization energy I of the molecules.

In this article data are presented on I, T_1 and S_1 for 18 condensed ring aromatic hydrocarbons and for 10 methyl derivatives of 1 : 2-benzanthracene. The latter were included in the study in order to increase the number of carcinogenic hydrocarbons considered.

The energy E_{CT} of maximum absorption of the charge-transfer band of the complex with chloranil in carbon tetrachloride solution has been observed for each compound. Similar measurements on some of the compounds have been reported by previous observers[11,13]. Our values agree closely with these, except in the case of tetracene and chrysene, where they confirm the values of Briegleb and Czekalla[11], but are less by 0·11 eV. and 0·23 eV. respectively than those of Peticolas[13]. I has been evaluated from the linear relation established by Foster[12] from observations on compounds of known I, namely:

$$I = 5{\cdot}13 + 1{\cdot}12\,E_{CT} \quad \text{(eV.)} \qquad (1)$$

Table 1. Condensed Ring Aromatic Hydrocarbons
(Energies in eV.)

		E_{CT}	I	T_1	S_1	$\bar{E}_1$	Carcinogenicity
1	Benzene	3·64	9·21	3·64 (ref. 6)	4·71	4·17	
2	Naphthalene	2·65	8·10	2·64 (ref. 6)	3·88	3·26	
3	Anthracene	2·00	7·37	1·82 (ref. 6)	3·32	2·57	
4	Tetracene	1·67	7·00	1·27 (ref. 14)	2·63	1·95	
5	Phenanthrene	2·64	8·09	2·68 (ref. 6)	3·58	3·13	
6	1:2-Benzanthracene	2·06	7·45	2·05 (ref. 16)	3·22	2·63	+
7	Chrysene	2·38	7·80	2·43 (ref. 15)	3·45	2·94	
8	Pyrene	2·16	7·55	2·09	3·34	2·71	
9	Triphenylene	2·64	8·09	2·88	3·54	3·21	
10	1:2:5:6-Dibenzanthracene	2·38	7·80	2·27 (ref. 16)	3·14	2·70	+ +
11	3:4-Benzpyrene	1·83	7·19	1·82 (ref. 6)	3·08	2·45	+ + + +
12	1:2:7:8-Dibenzanthracene	2·28	7·68	2·14	3·14	2·64	+
13	1: 2-Benzpyrene	2·17	7·56	2·30	3·22	2·76	
14	1:2:3:4-Dibenzanthracene	2·21	7·61	2·20 (ref. 6)	3·30	2·75	
15	Picene	2·38	7·80	2·62	3·30	2·96	
16	Perylene	1·70	7·03	1·55	2·85	2·20	
17	1:2:4:5-Dibenzpyrene	1·91	7·27	1·85	2·98	2·41	
18	Coronene	2·06	7·44	2·36 (ref. 16)	2·88	2·62	

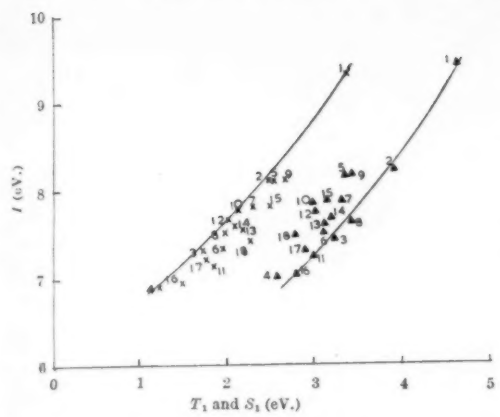

Fig. 1. Condensed ring aromatic hydrocarbons. T_1 (×) and S_1 (▲) against I

The triplet energy T_1 of seven of the compounds has been measured by the solvent perturbation technique[6] using oxygen under high pressure. The oxygen forms a contact charge-transfer complex with the hydrocarbon, and the absorption spectrum corresponding to the $S_0 \rightarrow T_1$ transition is observed superposed on the charge-transfer band. Values of T_1 for the other compounds have been obtained from the data of other observers who used solvent perturbation[6,14] or phosphorescence spectrometry[15–17]. Values of S_1 have been obtained from the solution absorption spectra[18,19].

The results are presented in Tables 1 and 2.

Table 2. Methyl Derivatives of 1:2-Benzanthracene (Energies in eV.)

	E_{CT}	I	T_1 (ref.17)	S_1	$\overline{E}_1$	Carcinogenicity
1:2-Benzanthracene	2·06	7·45	2·05	3·22	2·63	+
2'-Methyl	2·02	7·39	2·06	3·22	2·64	
3'-Methyl	2·05	7·43	2·07	3·23	2·65	
4'-Methyl	2·05	7·43	2·07	3·20	2·63	
3-Methyl	2·05	7·43	2·08	3·20	2·64	+ +
4-Methyl	2·03	7·41	2·10	3·23	2·66	+ +
5-Methyl	2·02	7·39	2·06	3·23	2·64	+ +
6-Methyl	2·00	7·37	2·05	3·22	2·63	+
7-Methyl	2·00	7·37	2·06	3·21	2·63	+
10-Methyl	1·92	7·29	2·02	3·20	2·61	+ + + +
9:10-Dimethyl	2·05	7·43	1·92	3·26	2·59	+ + +

For many of the compounds E_{CT} and T_1 are practically equal. This feature, which has been noted previously by Peticolas[13], is considered to be accidental and not of general significance, since in several of the compounds there are clear differences between E_{CT} and T_1. E_{CT} depends on the particular charge-transfer acceptor, chloranil, and quite different values of E_{CT} are obtained with benzoquinone and trinitrobenzene, although these yield similar values of I from equations analogous to (1) (ref. 12).

I is plotted against T_1 and S_1 in Fig. 1. There is a very approximate linear relationship between I and S_1, corresponding to the simple Hückel-type equation:

$$I = \alpha + bS_1 \qquad (2)$$

used by some authors[20] to estimate I. The degree of scatter in the experimental data indicates the inaccuracy of this method of obtaining I. There is a similar approximate linear relationship between I and T_1, which reflects the similarity between E_{CT} and T_1 in many of the compounds.

A more significant feature is that the curves through the values of S_1 and T_1 for the polyacenes (compounds 1–4) form upper and lower limits respectively for the values of S_1 and T_1 for the other compounds. For a given I, non-linear molecular arrangements of the aromatic ring systems yield lower or equal values of S_1 and higher or equal values of T_1 than the linear polyacene arrangement. Expressed alternatively, for a given T, the S_1–T_1 separation, corresponding to the spin-reversal energy, is greatest for the polyacenes.

These considerations suggest that the mean first π-excitation energy:

$$\overline{E}_1 = \tfrac{1}{2}(T_1 + S_1) \qquad (3)$$

is a more meaningful quantity to compare with I, when considering condensed-ring molecules of different shapes. I is plotted against $\overline{E}_1$ in Fig. 2. A clear linear relationship is found between I and E_1 given by:

$$I = 4{\cdot}39 + 1{\cdot}15\,\overline{E}_1 \qquad \text{(eV.)} \qquad (4)$$

which is obeyed, within experimental error, by all except three of the compounds. Moreover, the data extrapolate to give an intercept of 4·39 eV. for graphite, the infinite condensed ring 'molecule', at $\overline{E}_1 = 0$, in agreement with the experimentally determined work function[21].

The agreement of the experimental data with (4) is sufficiently striking to consider possible causes for the discrepancies in three of the compounds. The compounds in question, tetracene, 1 : 2 : 5 : 6-dibenzanthracene and 1 : 2 : 7 : 8-dibenzanthracene, possess the longest molecules of those considered, and it is possible that the deviations are due to

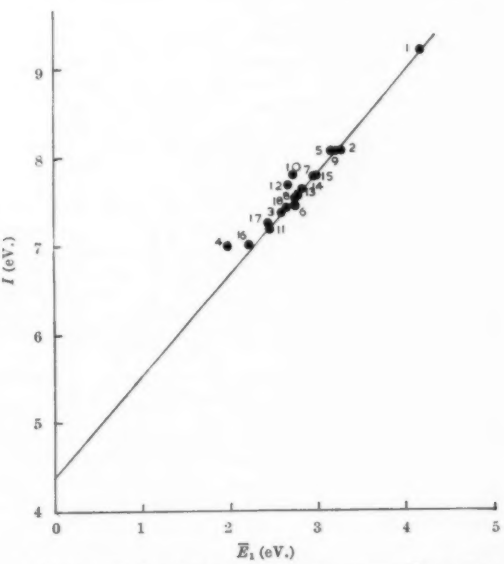

Fig. 2. Condensed ring aromatic hydrocarbons. $\overline{E}_1$ against I

the molecule being too elongated. It is of interest to note that molecular orbital theory[2] indicates that the lowest singlet state in the polyacenes changes from $^1B_{2u}^-$ to $^1B_{2u}^+$ on increasing the number of rings to 4 or more, and, while yielding reasonable values for the lower polyacenes, predicts a higher value for S_1 (3·11 eV.) for tetracene than that observed. The use of this value for S_1 would increase $\overline{E}_1$ to 2·20 eV. for tetracene and remove the anomaly. There has been some controversy over the value of T_1 for tetracene, but the experimental value taken is consistent with those of the lower polyacenes and with theory[2,16]. A further possibility is that deviations from the linear relation (1) may occur with elongated molecules.

The other two compounds have not been studied theoretically in any detail. They each have S_1 = 3·14 eV. and S_2 = 3·52 eV. The anomalies could be removed by using S_2 rather than S_1 in (3); but there appears little justification for this arbitrary procedure.

The measurements on the condensed-ring compounds and on the methyl derivatives of 1 : 2-benzanthracenes show that of the various energies observed only S_1 shows any degree of correlation with the carcinogenicity. This correlation has been discussed previously[22].

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ELECTRICAL EFFECTS DURING DISSOLUTION AND PRECIPITATION OF ELECTROLYTES

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EVIDENCE, although scant, has accumulated during the past three decades which indicates that potential differences are produced during phase-transformations[1-4]. Unfortunately, the phenomena associated with such transformations have not been subjected to systematic study. Hence, we have only doubtful explanations regarding the origin, build-up and the decay of potential difference. Difficulties in making reproducible measurements of potential differences are considerable. The purpose of the present article is to report the study of potential differences that are produced when the crystals of an electrolyte just appear or when these are allowed to dissolve. We will call the former the precipitation potential, and the latter the dissolution potential. So far as we are aware, this has not been reported earlier.

The initial experiments for measuring potential differences were performed in a simple way. A copper or silver tube was immersed in a bath kept at a certain temperature below the saturation temperature of the solution. This tube served as one electrode. A piece of wire of the same metal was kept in the centre of the tube, serving as another electrode. The two electrodes were connected through a Pye Scalamp galvanometer and a megohm resistance box having various steps in series. By adjusting the resistance and knowing the sensitivity of the galvanometer it could be converted into a direct-reading milli-voltmeter of high input impedance. This was considered to be sufficient for the purpose, since the saturated solutions of electrolytes are sufficiently conducting and the electrode area was quite large. The saturated solution was poured into the tube and the central electrode was immediately inserted. Potential difference was measured instantaneously. The experiments were performed with aqueous solutions of potassium chloride and potassium iodide saturated at 40° C. The asymmetry potential and thermo-e.m.f. were estimated and found to be negligible compared with the observed maximum potential differences. The decay of potential difference as a function of time was also observed. Smooth curves were obtained. The decay curves obtained by Workman and Reynolds for the freezing of ice were erratic, most probably due to difficulty in avoiding solidification on both the electrodes simultaneously. In our experiments one of the electrodes was always clean.

Difficulties in these experiments are numerous. First, the temperature corresponding to maximum potential difference is difficult to estimate, owing to the fact that nucleation may take place at any temperature intermediate between the saturation temperature and the bath temperature. Secondly,

it was realized that owing to large electrode area, there was a distinctly greater possibility of nucleation occurring in crevices. This was quite clear, since great difficulty was encountered in cleaning the electrodes after each experiment so as to give negligible asymmetry potential.

These difficulties were removed to a great extent in subsequent experiments. The saturated solution was kept in a 'Pyrex' test-tube, closed at the upper end by a cork through which two electrodes, of good connecting wire, passed. One of the electrodes was in the form of a spiral. This almost touched the bottom of the tube. The other electrode just touched the surface of the solution. This was done to ensure that deposition of the crystals did not occur simultaneously on the two electrodes. If the nucleation or crystallization occurs on the two electrodes then the potential difference is neutralized or obscured. To guard against this, the second electrode was only inserted when the observations were to be made. Potential difference was measured with a vacuum tube voltmeter giving values that were correct to $\pm$ 5 mV. The 'Pyrex' tube was kept in a large beaker containing water. The outer bath was cooled in a controlled way. The cooling-rate was about 1 deg. in 5 min. The fall in temperature of the bath was followed closely by the aqueous solution of electrolytes in the 'Pyrex' tube. As soon as the first crystals appeared on the electrode, the potential difference was noted. The temperature of the bath was also noted at the same time. The crystallization at any desired temperature was induced by seeding. In this manner, the potential differences for a number of electrolytes were noted for different degrees of undercooling. To give an idea of the magnitude of the effect, only maximum values are shown in Table 1.

Table 1. PRECIPITATION AND DISSOLUTION POTENTIALS FOR ELECTROLYTES

(For precipitation potentials, the saturation temperature was 50° C.)

Substance	Precipitation potential		Dissolution potential	
	Temperature (°C.)	Potential difference (mV.)	Temperature (°C.)	Potential difference (mV.)
Potassium bromide	41	115	70	505
Potassium nitrate	44	120	80	184
Sodium acetate	42	460	70	350
Potassium acetate	47	640	70	440
Ammonium chloride	43	155	70	320
Potassium chloride	45	220	70	206
Potassium iodide	40	145	70	750
Ammonium bromide	40	350	70	450
Oxalic acid	45	450	50	196

The reverse effect involving measurement of dissolution potential was also studied in a simple way. Silver wires were used as electrodes. One of the electrodes was kept in a test-tube in which aqueous solution of the electrolyte saturated at 70° C. or 80° C. was kept. On cooling to room temperature or even lower, the electrode was covered with a thick deposit of freshly prepared crystals. The tube was broken at the bottom, so that excess of water was removed. Now, the broken tube along with the electrode was dipped in water or in the solution of the same electrolyte at a definite concentration. The other electrode was kept in water or in the solution beforehand. The two electrodes were connected to a direct-reading millivoltmeter made from a Pye Scalamp galvanometer as described earlier. As soon as the electrode embedded with crystals was dipped, an instantaneous deflexion in the galvanometer was observed. A large number of electrolytes were investigated. The effects of temperature, concentration and stirring were also investigated. The maximum values of dissolution potentials observed in water are also given in Table 1.

The following conclusions can be drawn from the data collected in the course of this work:

(1) The potential difference is developed as soon as a tiny crystal is formed at the electrode. However, we cannot say whether the potential difference is developed when the crystal nuclei is just formed or when it grows to certain dimensions. In the same manner, dissolution potential is developed as soon as the first crystals go into the solution.

(2) The electrode at which the crystallization takes place was always found to be positive. Similarly, in the case of dissolution potential, the electrode on which the dissolution took place was found to be positive.

(3) The precipitation potential increases with the degree of undercooling. The dissolution potentials also increase systematically in most cases as the temperature of the water is increased.

(4) The dissolution potential of an electrolyte in an aqueous solution of the same is a function of concentration. Its value increases as the dilution is increased.

(5) As the crystallization or dissolution proceeds, the potential difference diminishes. The decay curve of potential difference would be erratic when the crystals are deposited simultaneously on the two electrodes or when the dissolution takes place from both electrodes.

(6) Both phenomena are independent of the nature of the electrodes.

The exact mechanism of these phenomena is difficult to establish at present until more information is available. It seems reasonable to assume that an electrical double-layer is formed when the solid phase is just formed or a fresh double-layer is regenerated when the solid just dissolves. The concept of electrical double-layer may be useful here, since it is able to explain adequately a number of interfacial phenomena[5]. But, if so, the electrical potential difference should be developed at the appearance of first crystal nucleus. It is difficult to provide evidence for it.

The results show clearly that the greater the rate of transformation, the greater is the potential difference developed. B. Gross[6] has developed a theory of such phenomena which relates the potential differences to the rate of transformations. According to his model, as the interface advances, it leaves behind a trail of space-charge in the solid between the region deep in the solid and the liquid, which builds up a potential difference. However, it is difficult to imagine how these charges are dissipated since ultimately the solid must be electrically neutral. Moreover, in the present case the electrical conductivity of solid electrolyte and the diffusion of the electrolyte in the solution would be important factors which are not taken into account by this theory.

More detailed investigations are in progress and may be published in due course. This work forms part of a project sponsored by the Indian Council of Scientific and Industrial Research.

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METAL CHLORIN COMPLEXES IN RECENT SEDIMENTS AS INITIAL PRECURSORS TO PETROLEUM PORPHYRIN PIGMENTS

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THE presence of the isocyclic ring in the porphyrins found in crude oil is usually regarded as indicative of a chlorophyll origin for the petroleum porphyrin–metal complexes[1]. Since it appears, therefore, that pigments derived from chlorophyll probably accompany the general organic matter in its conversion to crude oil, it is of interest to establish the path by which chlorophyll becomes transformed to petroleum porphyrin pigments. In this manner it may be possible to define the chemical environments of the formation of crude oil more clearly.

Chlorophyll is readily converted to phæophytin by the loss of magnesium[2]. However, the path by which phæophytin is converted to metal–porphyrin complexes is not well defined; as discussed in an earlier communication[3], it may involve one of two major intermediates. In one, the phæophytin forms a metal–phæophytin complex, a chlorin complex, which is afterwards reduced and dehydrogenated to a metal-containing porphyrin. In the other, the phæophytin is reduced and dehydrogenated to a free porphyrin which afterwards forms a complex with a metal. Heretofore, however, neither chlorin complexes nor free porphyrins have been reported in recent sediments.

Recently, however, a porphyrin–metal complex has been found in post-glacial lake sediments, having light-absorption maxima at 410, 536 and 575 mμ (ref. 3). There is evidence that it is a carboxylated porphyrin complex, only one step removed from the decarboxylated porphyrin–metal complexes of crude oil.

The purpose of this article is to report the results of attempts to close the gap between the free chlorins, which are abundant in potential petroleum source sediments, and the porphyrin–metal complex, which exists in sediments geologically very little older. Present chemical work in this laboratory shows the likely initial steps, and these are confirmed by new pigment discoveries in recent lake and delta sediments.

From considerations of the reactivity of the peripheral groups on the chlorophyll molecule, it appears reasonable to believe that the main reaction path would include phæophytin, phæophorbide, phæoporphyrin, phylloerythrin, and deoxophylloerythrin, leading to deoxophylloerythroetioporphyrin of crude oil. A series of experiments subjecting phæophorbide to extended thermal action in a methanol–water system at 180° C. appeared to confirm such a sequence in principle. Compounds essentially similar to phæoporphyrin and phylloerythrin occurred within a few hours, followed shortly by two deoxophylloerythrin-like substances. Identification of the compounds was based on acid-solubility characteristics in addition to spectral configurations which have been established in this laboratory for the known compounds prepared according to Fischer[4]. Thus, the evolution of such compounds in a relatively simple thermal system indicates that such a pathway may be possible in this particular metal-free environment.

However, free porphyrins in potential petroleum source sediments have not been reported, and present work in this laboratory has as yet failed to reveal any. Such negative findings, therefore, tend to direct attention to the other path, namely, that involving the formation of a metal–chlorin complex which is afterwards transformed to a metal–porphyrin complex.

To investigate this point, various chlorophyll-containing mixtures of either spinach leaves or commercial chlorophyll were allowed to react with various vanadium salts in the presence of sodium sulphide in aqueous systems at temperatures ranging from 90° to 150° C. In most cases, starches, sugars and proteins were included as well, further to simulate source sediment environments. Vanadium complexes of various pigments appeared in the reaction systems after several hours, with the major pigment classes characterized by absorption spectra maxima at about 650 and 620 mμ for one, and 650 and 590 mμ for another. The first appeared to be a precursor of the second. A further reaction of phæophytin *a* with ammonium metavanadate in the presence of only sodium sulphide and an unsaturated vegetable oil in an aqueous system at 105° C. produced the same two classes of compounds in substantial yields in 60–70 hr. Part of each class of compounds showed the presence of free carboxyl groups, indicating at least partial hydrolysis of the phytyl alcohol group from the phæophytin. These compounds were insoluble in 6 *N* and 12 *N* hydrochloric acid and were, therefore, regarded as metal complexes of the pigments, presumably vanadium complexes. The location of spectral peaks indicated complexes of chlorins rather than porphyrins, since the latter absorb in a significantly lower spectral range[5].

Similar chlorin pigments were evident in recent sediments obtained from widely differing environments. For example, sediments from Lake Wabamun, a large, relatively shallow lake about 40 miles west of Edmonton, Alberta, Canada, were exhaustively extracted with aqueous acetone and the extracts transferred to ether. Uncomplexed chlorin pigments, principally phæophytin *a* and phæophorbide *a*, were acid-extracted from the ether, leaving an appreciable amount of chlorin-like material along with carotenoids and other substances. The acid-insoluble material, which would include any metal complexes of chlorins or porphyrins present in the sediments, was transferred to *n*-hexane and chromatographed on cellulose fibre.

Good spectral evidence was obtained for three pigments which were apparently metal complexes as indicated by both their acid-insolubility and spectral characteristics. Absorption maxima for the three compounds were : (*a*) 403, 598 and 648 mμ; (*b*) 413, 589 and 628 mμ; (*c*) 411 and 648 mμ.

The general spectral appearances were those of metal chlorins, similar to those of the products obtained in the vanadium–phæophorbide reactions reported above, and to that of the nickel phæophytin reaction previously reported at 410 and 635 mμ (ref. 6).

Analyses for vanadium, nickel and copper showed positive results for both vanadium and nickel. Material balances were difficult to test because of the lack of precise knowledge of the extinction coefficients of the pigments, and the incomplete separation of organic matter which was characterized by strong absorption below 500 mμ. Nevertheless, using the known extinction coefficient for the nickel complex of phæophytin[6], it was possible to show the presence of enough of both vanadium and nickel to account for the apparent amount of chlorin present. Thus, it is clear that recent sediments contain not only free chlorins but also metal-complexed chlorins. Accordingly, it is reasonable to suggest that the overall reaction path for the formation of petroleum porphyrin–metal complexes involves the entry of the metal into the pigment at a very early stage, the chlorin stage, in the general development of the pigment.

It is of some significance to note that the occurrence of metal–chlorins appears to be quite widespread. Metal–chlorin compounds very similar to those of Lake Wabamun, a plains lake, were found in a deep mountain lake, Pyramid Lake, in Jasper National Park, in the sediments of the delta of the Mackenzie River in the Canadian Arctic, and in the sediments of Laguna Madre in the Gulf of Mexico.

A further observation in the Lake Wabamun sediments is worthy of considerable attention. The sediments on the surface of the lake bottom contained not only metal complexes of chlorins but also, in addition, a metal complex of a porphyrin. It occurred in much smaller concentrations than the chlorin complexes, and had absorption maxima at 410, 530 and 570 mμ. Alkali solubility indicated the presence of a free carboxyl group. Another slightly different porphyrin complex had been found in a nearby lake as previously reported[3], but the significant difference is that in the present case the sediments were surface sediments, very recently deposited, in contrast with the deeper sediments which were probably several thousand years old.

In summary, there is much evidence to indicate that the reaction path for the formation of petroleum metal–porphyrin pigments involves the introduction of the metal into the pigment at a very early stage of development, the chlorin stage, and that almost the entire process is completed in a very short period of time; only hundreds of years are required for the deposition of a few inches of lake sediments.

We are pleased to acknowledge the assistance of R. H. Harvey and other skin-divers of the Edmonton Aquanauts for obtaining the lake-bottom sediments, G. H. Johnston, of the National Research Council, for the delta samples, Gene Rusnak, at present of the Marine Laboratory, University of Miami, for the sediments of Laguna Madre, and B. L. Baker, R. M. Elofson, and Brian Hitchon for general assistance and guidance.

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HISTOCHEMICAL DEMONSTRATION OF GAMMA-GLUTAMYL TRANSPEPTIDASE

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In a previous paper two of us[1] described the occurrence of a specific peptidase in blood serum and some tissues. The enzyme hydrolysed some α-(N-γ-DL-glutamyl)-aminonitriles and was suspected to be a γ-glutamyl transpeptidase, similar to that described by Hanes *et al.*[2]. Further work confirmed this supposition and recently[3] a new method was described for the determination of γ-glutamyl transpeptidase, using γ-L-glutamyl-α-naphthylamide as substrate. The enzyme catalyses with this substrate a transpeptidation reaction according to the following scheme:

2γ-L-glutamyl-α-naphthylamide → α-naphthylamine + γ-L-glutamyl-γ-L-glutamyl-α-naphthylamide

The amount of α-naphthylamine liberated during the enzymic reaction was estimated by a modification of the Bratton and Marshall procedure[4] and used as a measure of enzyme activity. With this method, the enzyme activity was found to be very high in human kidney. Much lower activities were found in human liver, spleen and pancreas. Some activity was found in human serum and urine[3].

This article deals with the histochemical demonstration of γ-glutamyl transpeptidase in tissue sections of some animals and human beings. γ-L-glutamyl-α-naphthylamide was used as substrate and the liberated α-naphthylamine was coupled to an azo-dye with the aid of fast garnet *GBC* salt. The composition of the incubation mixture was as follows: 6 ml. substrate solution (5·44 mgm. γ-L-glutamyl-α-naphthylamide dissolved in 0·9 per cent sodium chloride), 4 ml. buffer solution (0·1 *M* phosphate buffer *p*H 6·7, or 0·2 *M* citrate–phosphate buffer at the same *p*H), 5 mgm. of fast garnet *GBC* salt per 10 ml. of incubation mixture. This incubation mixture was prepared fresh daily. Good results were also obtained using γ-L-glutamyl-β-naphthylamide. However, the solubility of this compound is very low at *p*H 6·7, and even 0·2 mgm. of this substrate per ml. of incubation mixture was difficult to dissolve. The hexazonium salt of pararosanilin[5] was also used as coupling agent instead of fast garnet *GBC*. Places

with γ-glutamyl transpeptidase activity exhibit a brown-reddish colour with fast garnet *GBC* or a yellow colour with the hexazonium salt of pararosanilin as coupling agent.

The optimum *p*H for γ-glutamyl transpeptidase is about 8·8 and at *p*H 6·7 the enzyme activity amounts only to a few per cent of that measured in optimal conditions. Acceleration of the enzymatic reaction could be obtained at *p*H 6·7 by the addition of glycylglycine, which serves as an acceptor of the γ-glutamyl moiety according to the following reaction[3]:

$$\gamma\text{-L-glutamyl-}\alpha\text{-naphthylamide} + \text{glycylglycine} \xrightarrow{\text{enzyme}} \gamma\text{-L-glutamyl-glycylglycine} + \alpha\text{-naphthylamine}$$

For acceleration of the histochemical reaction, we have added 20 μmoles of glycylglycine per ml. of incubation mixture. The addition of glycylglycine is particularly recommended for the demonstration of γ-glutamyl transpeptidase in tissues with low enzyme activity.

Healthy mice, rats, guinea pigs and rabbits were used for our experiments. Moreover, kidney sections were taken from human subjects some hours after death in street accidents. Sections, 3–4 mm. thick, were prepared from kidneys, spleen, liver, pancreas, testes, myocardium and lungs, and fixed in acetone, absolute ethyl alcohol or 6 per cent neutralized formalin. The fixation time was 11 hr. at 4° C. As freezing with carbon dioxide gave inferior results, the sections were embedded in paraffin. 5–10μ thick sections were incubated in the given mixture for varying periods of time. 5–20 min. were sufficient for kidney while for other tissues an incubation time of 1–3 hr. was necessary. Following incubation, the slides were immersed for 2 min. in 0·1 *M* solution of copper sulphate and then stained with hæmatoxylin for 1–2 min. Afterwards, they were rinsed with 0·9 per cent sodium chloride and mounted in glycerol–gelatin.

The following localization of the enzyme was observed. In the kidney, the lumen of the loop of Henle and to a less extent of the proximal convolutions of the tubulus shows homogeneous masses with a strong positive reaction. The enzyme was not visible in the glomeruli and collecting tubules. In mouse and rat kidneys the enzyme is distributed quite regularly all over the cortex (Fig. 1) whereas in guinea pigs and rabbits γ-glutamyl transpeptidase is located almost exclusively in medullary rays (Fig. 2). The localization of the enzyme in normal men is similar to that found in rabbits and guinea pigs. It is to be stressed that the homogeneous masses with a strong positive reaction adhere to the surface of epithelial cells not only in proximal convolutions of the tubulus, which possess a distinct brush border, but also in the loop of Henle devoid of this brush border.

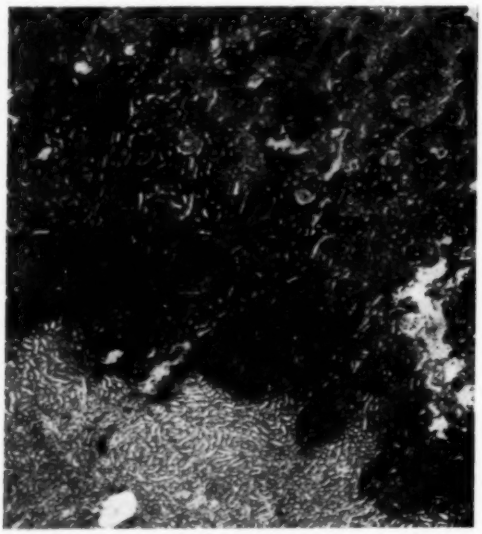

Fig. 1. γ-glutamyl transpeptidase in the cortex of the mouse kidney. (× 42)

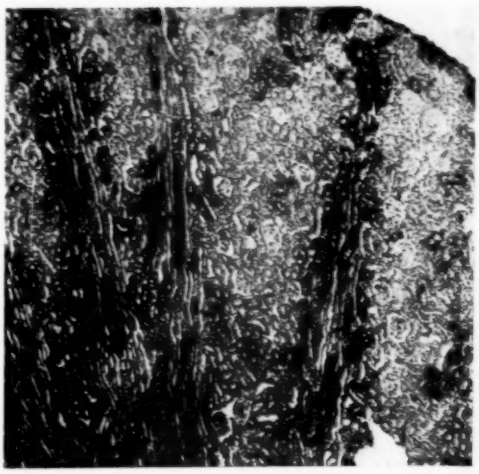

Fig. 2. The localization of γ-glutamyl transpeptidase in medullary rays in the kidney of the guinea pig. (× 42)

With the spleen, the enzyme is visible in guinea pigs, rabbits and rats in the walls of some capillaries of the red and white pulp, and moreover as granules in some cells of the reticulo-endothelial system.

With the liver, a positive reaction was seen in rats in the walls of bile canaliculi and in single cells of the reticulo-endothelial system in Glisson's capsule. In rabbits the enzyme was found in the form of granules in the cytoplasm of liver cells mostly around the nucleus. It was more abundant in the periphery than in the centre of lobules. The greatest enzyme activity was found in guinea pigs, taking the shape of confluent tiny granules on the periphery of liver cells and, as in rabbits, more profuse in the peripheral than the central part of the lobules.

With the pancreas, a relative abundance of the enzyme was seen in the cells of the outer secretory system in the mouse, rat, rabbit and guinea pig. The enzyme was not found in Langerhans's islands.

With testes, a positive reaction was observed in the testes of guinea pig within spermatozoa and spermatocytes. No activity was seen in the testes of the rat and the mouse. The myocardium and lungs of the mouse, rat and guinea pig did not exhibit enzyme activity.

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REACTIONS OF HÆMOPROTEINS WITH HYDROGEN PEROXIDE AND THE SUPPOSED FORMATION OF HYDROGEN PEROXIDE DURING THE AUTOXIDATION OF HÆMOGLOBIN

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IT is well known that many substances undergoing autoxidation, or oxidation catalysed by metals, or by some specific enzmyes, reduce molecular oxygen, directly or indirectly, to hydrogen peroxide, which can usually be detected by some simple reagents. However, the main limitations of these reagents are that they can seldom be used for the study of the continuous formation of hydrogen peroxide and that they may interfere with the primary oxidation reactions which generate the peroxide, especially if these reactions are brought about by some unstable catalytic systems. In such cases peroxidase, catalase and methæmoglobin can be used both for the detection of hydrogen peroxide and for following its gradual generation even by very unstable biological systems. These three hæmoproteins show striking resemblances in their structure and properties, including some of their reactions with hydrogen peroxide[1–4].

Reactions of Hæmoproteins with Hydrogen Peroxide

The following few examples illustrate the reactions of hæmoproteins with hydrogen peroxide generated by an oxidation system.

(1) *Catalatic decomposition of hydrogen peroxide.* The reduction of molecular oxygen to hydrogen peroxide by glucose oxidase (notatin), which catalyses the oxidation of glucose to gluconic acid, can easily be followed manometrically[5]. Thus, catalase added to this system, by liberating oxygen from the peroxide, halves the original uptake of oxygen and brings it down to the theoretical value corresponding to the reduction of oxygen to water. Similar results have been obtained with many other oxidizing systems.

(2) *Peroxidatic utilization of hydrogen peroxide.* Of special interest in this respect was the discovery that catalase, in addition to its well-known property of decomposing hydrogen peroxide to oxygen and water, is also capable of utilizing the hydrogen peroxide generated for the peroxidatic oxidation of alcohol to aldehyde[4,5]. The concept of the peroxidatic activity of catalase was later extended to some other hydrogen donors, including hydrogen peroxide itself[6–8]. A large variety of hydrogen donors were found to undergo peroxidatic or coupled oxidation catalysed by peroxidase, catalase and methæmoglobin. These hæmoproteins, however, show marked differences in their specificity and in their catalytic efficiency towards hydrogen donors[5]. As some of these donors, like benzidine, give highly coloured products on peroxidatic oxidation catalysed by peroxidase and methæmoglobin, the presence of hydrogen peroxide can easily be detected by this well-known colour reaction.

(3) *Compounds of hæmoproteins with hydrogen peroxide.* As these hæmoproteins form with hydrogen peroxide compounds which are easily recognized by their colour and absorption spectra, they can be used for the detection of hydrogen peroxide, as reagents acting in the absence of hydrogen donors. While peroxidase and methæmoglobin can be used for this purpose in their free state, catalase gives the best result when it is used as the azide-catalase complex which, when added to a solution containing hydrogen peroxide, changes its colour and the pattern of its absorption spectrum[1,3]. Moreover, azide catalase enables one to follow the formation of hydrogen peroxide even during the oxidation of sodium dithionite ($Na_2S_2O_4$) when other reagents cannot be used.

Is Hydrogen Peroxide formed during the Autoxidation of Hæmoglobin?

Oxyhæmoglobin, like hæmoglobin, is a compound with divalent iron atoms which may undergo slow autoxidation to the tervalent iron atoms of methæmoglobin. It was shown that up to 2 per cent of endoerythrocytic mammalian hæmoglobin *in vivo* may be present as methæmoglobin[9]. The hæmoglobin of *Gasterophilus* larvæ[10] and of certain parasitic nematodes[11], as well as myoglobin, are much more susceptible to autoxidation, especially during their purification, than are the mammalian blood hæmoglobins.

Of great interest, however, is the fact that while the oxidation of oxyhæmoglobin to methæmoglobin by potassium ferricyanide liberates all the bound oxygen, that is, 1 mole oxygen per gram-atom of iron, the autoxidation of oxyhæmoglobin liberates less than 50 per cent of the bound oxygen, instead of 75 per cent had some oxygen been used only for the oxidation of the divalent iron atoms of hæmoglobin. Brooks[12], who was the first to study the kinetics of the autoxidation of hæmoglobin, showed that the rate of this reaction at different oxygen pressures is proportional to the concentration of deoxygenated hæmoglobin. The observed first-order rate constant for the autoxidation of hæmoglobin is a function of the oxygen pressure, and has a well-defined maximum at about 20 mm. The kinetics and the possible mechanism of this reaction have recently been studied by several workers, both for hæmoglobin and myoglobin. The autoxidation is greatly accelerated by lowering the *p*H to about 5 and raising the temperature to 39°. Denaturation of oxyhæmoglobin by acid or alkali, which brings about the immediate oxidation of hæms to acid and alkaline hæmatin respectively, is also accompanied by the liberation of only a certain fraction of the bound oxygen.

It was suggested[13] that a part of the bound oxygen, which is not liberated from oxyhæmoglobin during its autoxidation, is activated and reacts with globin to form hydrogen peroxide which can be used by hæmatin for the catalysis of the peroxidatic oxidation of substances like ascorbate, bilirubin and biliverdin.

XUM

Unfortunately, no proper control experiments have been carried out on the oxidation of these substances by a solution of a parahæmatin or hæmochromogen in the absence of peroxide. The autoxidation of myoglobin was found to be accompanied by an uptake of 2·5 moles oxygen for each mole of metmyoglobin formed[14]. It is now generally accepted that the autoxidation of hæmoglobin and myoglobin to methæmoglobin and metmyoglobin respectively, brings about the simultaneous oxidation of some hydrogen-donor groups on globin itself. Although the mechanism of these autoxidation reactions is not yet properly understood, several workers[13–15] postulated the important part played in these reactions by hydrogen peroxide and related free radicals.

One of the objects of the present work was to find whether the postulated formation of hydrogen peroxide during the oxidation of oxyhæmoglobin on mild acidification can be demonstrated by some of the reactions of hydrogen peroxide with hæmoproteins which were discussed in the first part of this communication.

For this purpose experiments were carried out on horse-blood oxyhæmoglobin purified by crystallization which was repeated three times; the oxidation of oxyhæmoglobin was followed spectroscopically and the liberation of oxygen was determined manometrically.

The main results of these experiments can be summarized as follows:

(1) Contrary to claims made by previous workers[15], the addition of catalase to oxyhæmoglobin, prior to mild acidification, made no difference either to the rate of its oxidation to methæmoglobin, or to the amount of bound oxygen liberated from oxyhæmoglobin; ethanol added to this system failed to undergo oxidation to aldehyde.

(2) Benzidine added to the solution of oxyhæmoglobin did not undergo oxidation to a dark blue product on mild or strong acidification of this solution. The reaction failed even when a strong solution of purified peroxidase was mixed with oxyhæmoglobin. Yet, on the addition of traces of hydrogen peroxide to this solution, benzidine was rapidly oxidized to a dark blue product.

(3) Methæmoglobin formed during mild acidification never showed the presence of hydrogen peroxide by the formation of the methæmoglobin–peroxide compound, which could easily have been recognized by its red colour and the pattern of its absorption spectrum. The same applied to peroxidase added in a suitable concentration to an oxyhæmoglobin solution prior to its acidification.

(4) About 0·5 ml. of solution of pure peroxidase ($0{\cdot}37 \times 10^{-3}$ *M*) enclosed within a small dialysing sac, and showing its characteristic absorption spectrum, was immersed in 20 ml. of solution of oxyhæmoglobin ($1{\cdot}4 \times 10^{-3}$ *M* hæmin) which was acidified while being vigorously stirred. The dialysing sac, removed at different intervals of time for spectroscopic examination, failed to reveal any change in the absorption spectrum of peroxidase. Similar results were obtained when the dialysing sac contained a strong solution of azide catalase (in the presence of a large excess of azide) which is one of the most sensitive reagents for hydrogen peroxide.

All these experiments failed to give any indication of the formation of hydrogen peroxide in a solution of acidified oxyhæmoglobin undergoing autoxidation to methæmoglobin or to acid hæmatin. The problem of the mechanism of autoxidation of hæmoglobin and myoglobin therefore still remains open for further investigation.

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RISE OF ANTIBODY TITRE IN CELL-FREE SPLEEN PREPARATIONS INCUBATED *IN VITRO*

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AN initial attempt to demonstrate antibody-forming activity by cell-free tissue preparations was made by Šterzl and Hrubešová[1]. They injected cell-fractions and nucleoproteins from spleens of rabbits immunized with *Salmonella paratyphi B* vaccine into unimmunized neonatal rabbits and observed the appearance of specific agglutinins in the recipients. However, it is still not clear whether the antibodies appearing in the recipient animals were synthesized *de novo* after transfer or were initially bound to and afterwards released by the transferred materials[2]. With respect to the second possibility, a report by Feldman *et al.*[3] is of interest. They found that ribonucleoprotein-bound antibody was immunologically inactive but could be titrated following enzymatic degradation of the ribonucleoprotein.

The aim of the present work was to follow in an *in vitro* system the hæmolysin titres of extracts prepared from spleens of immunized rabbits. The animals were injected intravenously with standard doses of $0{\cdot}8 \times 10^9$ washed sheep erythrocytes/kgm. (equivalent to 1 ml. of a 5 per cent suspension/kgm.). Primary stimulation consisted of a single injection on days 0, 1 and 3, and secondary stimulation of a single dose given 8 weeks after primary injection.

The preparation of an experimental extract (series *A*) was as follows: five rabbits were killed by an

intravenous injection of air on day 4 or 5 following the first primary, or on day 3 following the secondary, injection. On these days, the serum hæmolysin titres of the animals ranged between 450 and 3,200 hæmolytic units/ml. The spleens of the rabbits were removed (average combined weight 9·4 gm.), cut into small pieces, and rinsed with about 5 ml. medium. The latter was a defined, complete tissue culture medium[4] with an amino-acid composition corresponding to that of rabbit γ-globulin[5]. For the purpose of these experiments, 0·7 μmole adenosine triphosphate[6] was added to the medium. The rinsed spleen pieces were combined with 1·0 ml. medium/gm. tissue and homogenized by 10 down–up excursions in a Potter–Elvehjem tissue grinder with a 'Kel-F' pestle. All procedures were carried out at 3° C. Probably not more than 75 per cent of the spleen cells were extracted by this method. This estimate is based on a count of the free nuclei present in the homogenate prior to centrifugation and the comparison of this number with the estimated number of cells representing 1 gm. of spleen tissue.

The homogenate was centrifuged at 500*g* for 10 min., and the resulting supernatant re-centrifuged at 1,700*g* for 15 min. The second supernatant (average volume 6·4 ml.), containing the large and small granule, and the soluble cell fractions, was transferred into a small Petri dish which was placed in a tissue culture incubator (37° C., 85 per cent humidity, 5 per cent carbon dioxide, 95 per cent air). The total time elapsed between homogenization and the beginning of incubation was 70–80 min. To avoid clotting, the extract was gently agitated with a magnetic stirrer during the second hour of incubation. Samples of 0·1 ml. were removed with ultramicro-pipettes at 0, 0·5, 1, 2, 3, 4, 6, 8, 12, 15 and 18 hr. Since it was found earlier that the samples did not contain detectable amounts of complement, complement inactivation was omitted.

The samples were diluted 1: 50 with veronal buffer and cleared at 1,700*g* for 20 min. Their hæmolysin titres, determined according to the method of W. H. and L. G. Taliaferro[7,8], were expressed in photometrically determined 50 per cent hæmolytic units/ml. extract. A 50 per cent unit is defined as the reciprocal of the amount of extract lysing 50 per cent of $3{\cdot}2 \times 10^8$ sheep erythrocytes in the presence of four 50 per cent units of complement. The over-all error of this titration method did not exceed 5 per cent.

Two types of controls were employed. The first (series *B*) involved the preparation of extracts from normal spleens to which hæmolytic serum was added immediately before homogenization. The amount of antibody added approximated the zero-time hæmolysin-level of the experimental extracts. The purpose of these controls was to determine the immunological stability of hæmolysins during *in vitro* incubation. The second set of controls (series *C*) was prepared from normal spleens alone.

Of nine experimental extracts, seven showed a significant elevation of hæmolytic activity, the net increase ranging between 21 and 99 hæmolytic units/gm. spleen. Because of 8-hr. intervals between samplings, one run could not be used for statistical evaluation, and only six of the positive experimental extracts are listed in Table 1 (*A*). Preparations from rabbits stimulated with primary or secondary antigen injections (four and two extracts, respectively) were considered together. The total incubation time was 8 hr. for four and 18 hr. for two of the series *A* extracts. Table 1 also lists the results obtained with five

Table 1. HÆMOLYTIC ACTIVITY DURING *in vitro* INCUBATION OF CELL-FREE SPLEEN PREPARATIONS

	A Experimental extracts (spleens of immunized rabbits)	*B* Control extracts (spleens of normal rabbits; immune serum added)
No. of extracts*	6	5
Combined spleen weight (gm.)	9·4 ± 0·4	7·0 ± 0·1
Volume of extract (ml.)	6·4 ± 0·7	5·5 ± 0·4
Hr. at beginning of titre rise above zero-time level	1·3 ± 0·4	(1·7 ± 0·9)†
Hr. of maximum titre (*A*) or of beginning of titre plateau (*B*)	7·3 ± 0·9	2·4 ± 0·2
Zero-time titre‡	128 ± 26	143 ± 34
Maximum titre‡	176 ± 32	148 ± 34
Net titre increase‡	48 ± 11	5 ± 5

Values given are means ± standard errors

* Each extract was prepared from the pooled spleens of five rabbits.

† The titres of only three of these extracts rose above zero-time level.

‡ In 50 per cent hæmolytic units/gm. spleen.

extracts from series *B* controls, three of which were incubated for 8 hr., and two for 18 hr. Not shown in Table 1 are three extracts of series *C* (normal spleens alone), since no hæmolytic activity could be detected during the 8-hr. incubation period.

Mean titration curves for series *A* and *B*, covering 8 hr. of incubation, are shown in Figs. 1 and 2. The graphs were obtained by calculating the mean hourly titres for a given series from the hæmolysin curves of the individual extracts, and plotting these means against time. Differences between certain parameters listed in Table 1 and corresponding ones of the

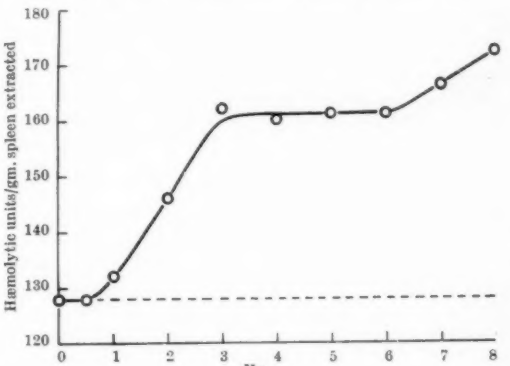

Fig. 1. Experimental extracts (series *A*). Mean hæmolysin curve of six *in vitro* incubated spleen extracts from rabbits immunized with sheep erythrocytes. (Each extract was prepared from five pooled spleens)

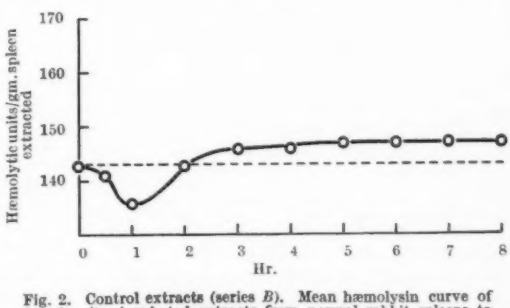

Fig. 2. Control extracts (series *B*). Mean hæmolysin curve of five *in vitro* incubated extracts from normal rabbit spleens to which hæmolytic serum was added before homogenization. (Each extract was prepared from five pooled spleens)

average curves are due to differences in the statistical approaches used to calculate the two sets of data.

The configuration of the mean hæmolysin curve for series *B* indicates a temporary immunological inactivation and subsequent reactivation of about 5 per cent of the added antibody. Inactivation may have been due to the adsorption of globulins to subcellular particles such as mitochondria and microsomes. The hæmolysins probably became detectable again after dissociation from these particles. Since adsorption may have begun during homogenization (70–80 min. prior to incubation), the initial titre of these extracts probably was at the level of the final titre plateau.

The two-step antibody rise shown by the mean hæmolysin curve for series *A* was displayed by five of the six extracts in this series. In some preparations, the initial antibody increase was followed by a shallow decline lasting 2 hr. or less. In the two extracts incubated for 18 hr., the second hæmolysin rise led to a plateau beginning at about 8 hr. which remained constant during the remainder of the incubation period.

The progressive increase in titre observed over a period of 8 hr. in spleen extracts from immunized animals suggests either the release of preformed antibody alone from a sub-cellular component, or the release of both preformed and newly synthesized antibody. The successive occurrence of these two processes may be indicated by the biphasic character of the mean hæmolysin curve.

I am indebted to Prof. Dan H. Campbell for his advice, and for supplying technical assistance and facilities.

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BIOSYNTHESIS OF RIBOFLAVIN IN CELL-FREE SYSTEMS

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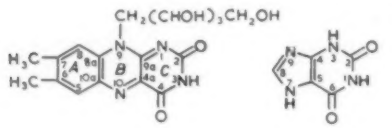
IT has now been well established that ring *C* and N-9 and N-10 of ring *B* of riboflavin (I) are formed from a purine (II) derivative following the loss of C-8 of the purine[1,2]. For example, Plaut[3] and his colleagues observed that the labelling pattern in riboflavin synthesized by the mould *Ashbya gossypii* fed carbon-14-HCO′$_3$, -H.COO′ and -glycine was identical with that observed in purines synthesized in the presence of the same labelled substrates, and similar results have been obtained using carbon-14-L-serine[4] and carbon-14-L-threonine[5]. Experiments with generally labelled carbon-14 adenine showed that incorporation did not first involve the degradation of a purine and then re-synthesis of the metabolites into riboflavin[6], and these results have been confirmed using [^{14}C : ^{15}N] xanthine and guanine[7].

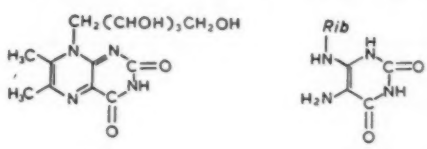


(I) (II)

Under suitable cultural conditions up to 70 per cent of added carbon-14 guanine is incorporated into riboflavin by the yeast *Candida flareri*[8]. C-8 of the purine is lost during incorporation, which would indicate that 4,5-diaminouracil, or more likely a derivative, is the intermediate[9]. Support for this view came when 4,5-diaminouracil was identified as a metabolite of the flavinogenic fungus *Eremothecium ashbyii*, but the methods employed in these experiments would not preclude its existence *in vivo* as a ribose phosphate or ribitol derivative[10]. This raises a central problem in elucidating the mechanism of biosynthesis. At what stage and how does the ribitol side-chain become attached to N-9? The problem has not yet been settled; all that can be said at the moment is that as 6-7-dimethyl-8-ribityllumazine (III) is a precursor of riboflavin (see below), the ribitol residue appears before the completion of ring *A*. The only hint of mechanism comes from the work of Forrest and McNutt[11] with generally labelled carbon-14 guanosine; they found that the riboflavin arising from this substrate was much more strongly labelled in the ring system than in the ribitol side-chain; this observation strongly suggests that the ribitol did not arise by reduction of the ribose residue. However, this cannot be accepted out of hand, because guanosine could be in equilibrium with guanine and ribose-1-phosphate (reaction 1) in the presence of a nucleoside phosphorylase, and if the pool size of ribose-1-phosphate were much larger than that of guanine (and this is highly probable because it is 'active glycine', and thus purine, which is the limiting factor in flavinogenesis in *E. ashbyii*), then this would lead to the observed results:

$$\text{guanosine} + Pi \rightleftharpoons \text{guanine} + \text{ribose-1-phosphate} \quad (1)$$

On the assumption that 4-ribitylamino-5-aminouracil (IV) might be the active precursor of riboflavin, a number of investigators synthesized this compound, which was found to be very unstable. Katagiri *et al.*[12]



(III) (IV)

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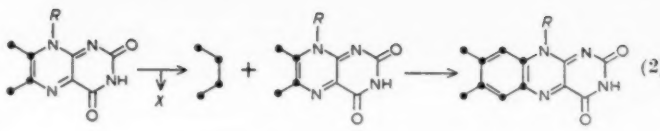
have reported a slight conversion of this compound into riboflavin in *Aerobacter aerogenes*, but Plaut, who has given a full chemical synthesis of the compound[13], has not yet reported on its biological activity and has indicated, in a personal communication, that his results were most inconclusive; Kishi *et al.*[14] also failed to demonstrate any significant production of riboflavin when they used a crude enzyme preparation of *Eremothecium ashbyii*. With the collaboration of Dr. B. G. Audley, we synthesized (IV), using essentially Plaut's methods, and consistently failed to observe its conversion by cell-free extracts of *Candida flareri* into either 6,7-dimethyl-8-ribityllumazine or into riboflavin, although many variations in preparing the extracts and in incubation conditions have been tried[15]. If (IV) were a precursor of riboflavin, then the experimental conditions necessary for the demonstration of precursor activity are extremely elusive.



(2)

However, investigations on the final stages of riboflavin biosynthesis have met with a little more success. Masuda in 1956 isolated and identified 6,7-dimethyl-8-ribityllumazine (III) from the mycelium of *E. ashbyii*[16]. He finally proved the structure by total synthesis[17]. Thanks to the generosity of Dr. Masuda, who kindly provided us with a crystalline specimen of (III), we were able to demonstrate that cell-free extracts (produced by sonic disintegration), but not intact cell suspensions, of *Candida flareri* rapidly converted it into riboflavin. The activity resided in the supernatant fraction (not sedimentable at 100,000*g*) of the extract and the maximum conversion obtained under many varying conditions was always approximately 30 per cent. It is interesting to record here that, with Dr. B. G. Audley, we found that distilled water (*p*H 6·0) was an excellent solvent for separating (III) from riboflavin on paper chromatograms. The presence or absence of possible sources of the 4-carbon unit (for example, pyruvate, acetate, acetoin) required for the completion of ring *A* had little effect on the conversion, and riboflavin was synthesized from (III) alone in extracts which had been dialysed for 18 hr. against running de-ionized water (20 litres). The extent of conversion in such dialysed extracts was also unaffected by addition of possible 4-carbon precursors, by 10^{-3} *M* fluoride, or by the addition of a mixture of ATP, coenzyme *A*, thioctic acid, thiamine, diphosphopyridine nucleotide, and magnesium and calcium ions. While this work was in progress, similar results were reported for extracts of other organisms, including *E. ashbyii*[12,18], *Ashbya gossypii*[18,19], *Escherichia coli*[12], *Neurospora crassa*[12], *Lactobacillus plantarum*[12], bovine liver[12] and *Mycobact.* spp.[18]. Also in agreement with our results, it was found that intact cells of *E. ashbyii*[18,20] and *A. gossypii*[19] failed to carry out the conversion; furthermore, extracts of a riboflavin-requiring mutant of *Lactobacillus casei*[12,21] also lack the enzyme(s) necessary for the conversion.

Consideration of our results led us to the view that the conversion of (III) into riboflavin must be some type of 'dismutation' in which one molecule of (III) yields a 4-carbon unit, or two 2-carbon units, which can combine actively with a molecule of unchanged (III) to produce riboflavin. This can be formally visualized as indicated in reaction (2) (there are other possibilities which will give the same overall result).

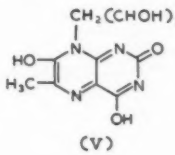
In order to test this hypothesis, we synthesized (III), labelled with carbon-14 in the positions indicated thus ● (see above), by treating 4-ribitylamino-5-aminouracil (IV) with generally labelled carbon-14 acetoin. The generally labelled acetoin was prepared enzymically by using a cell-free preparation (11,000*g* for 1 hr. at 4°) obtained from a sonate of *Acetobacter rancens* 23, which under appropriate conditions will convert pyruvate into acetoin in very high yield (80 per cent)[22]; when [2,3-^{14}C] pyruvate was used as substrate, generally labelled acetoin was obtained.

The 6,7-dimethyl-8-ribityllumazine, labelled with carbon-14 as indicated in reaction (2), was diluted with carrier 6: 7-dimethyl-8-ribityllumazine and added to a dialysed cell-free preparation of *Candida flareri*; after incubation under optimal conditions for conversion (28° for 3 hr. with constant shaking) the reaction was stopped, carrier riboflavin added and crystalline riboflavin isolated. The radioactivities in this and in the acetic acid obtained from C-6 and C-7 and the attached methyl groups by Kuhn–Roth degradation were assayed as previously described[5]. The results obtained and the values expected if reaction (2) obtains are given in Table 1. They are clearly consistent with the view that (III) liberates enzymically an active 4-carbon unit (or two 2-carbon units) which will combine with (III) to form riboflavin. It is interesting to find that Plaut had come to the same conclusion independently regarding the formation of riboflavin from (III), and he has reported recently an investigation with a partly purified enzyme system which indicates that three molecules of (III) disappear for every one molecule of riboflavin produced[23].

Table 1. ENZYMIC CONVERSION OF [^{14}C]-6,7-DIMETHYL-8-RIBITYLLUMAZINE INTO RIBOFLAVIN IN CELL-FREE EXTRACTS OF *C. flareri*. Dialysed cell-free extract of *C. flareri* used with no substrate other than [^{14}C]-6,7-dimethyl-8-ribityllumazine labelled as indicated in reaction 2; incubation for 3 hr. at 28° with continuous shaking

Compound	Specific activity (c.p.m./μmole) Expected	Observed
6,7-Dimethyl-8-ribityllumazine	—	8,000
Riboflavin	16,000	13,200
Acetic acid (from C-6 and C-7 and attached methyl groups)	3,300	3,000

During our experiments on the conversion of (III) into riboflavin, we encountered a secondary product, which shows up as a violet fluorescent spot on chromatography. This is clearly 'compound V' of Masuda *et al.*[24], which has the structure (V). There is general agreement that compound V is not an intermediate between 4-ribitylamino-5-aminouracil and riboflavin[25], but that it is formed as a side-reaction from (III), possibly by reaction of '*X*'



(V)

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(reaction 2) with pyruvate[23,24]. If this is so, then it would appear that pyruvate as such is not the condensing unit for production of the aromatic ring of riboflavin. However, it is fairly clear that acetate is not immediately concerned[25], and shikimic acid, the other well-known precursor of aromatic rings[2], is also not involved[27]. It seems likely then that pyruvate gives rise to 'activated acetaldehyde'[28,29], which is incorporated step-wise into (III), or which condenses to form a 4-carbon unit such as acetoin, which combines with (IV) to yield (III). In support of this view it was found that [^{14}C] acetoin ([1-^{14}C]-3-hydroxy-2-butannone) is incorporated into ring *A* of riboflavin with the expected labelling pattern[27]. In this connexion it should be emphasized that the carbon-14 acetaldehyde liberated from carbon-14 threonine by the enzyme threonine aldolase in extracts of *E. ashbyii* is not incorporated into ring *A* of riboflavin[5]; this means either that the acetaldehyde liberated is never 'activated' or that 'activated acetaldehyde' is not concerned with ring *A* synthesis.

Conversion of 6,7-dimethyl-8-ribityllumazine into riboflavin in rat liver. The fact that riboflavin arises in micro-organisms from purines raises an interesting problem regarding the nature of the metabolic lesion which makes animals dependent on exogenous riboflavin, because all living systems can synthesize the purine skeleton for incorporation into their nucleic acids. It was reported that (III) could be converted into riboflavin by bovine liver preparations[12]; we decided to re-investigate this, using rat liver homogenates. We found that the supernatant from such homogenates centrifuged at 25,000*g* contained a substantial amount of flavin adenine dinucleotide; on incubation this disappeared and free riboflavin appeared. Thus the very high blanks obtained because of the presence of an active flavin adenine dinucleotidase in our preparation made it impossible to decide whether (III) was converted into riboflavin or not. This can be decided finally only by using [^{14}C]-6,7-dimethyl-8-ribityllumazine followed by enzyme purification. Katagiri *et al.*[12] did not comment on this possibility of riboflavin formation in the report of their work on bovine liver extracts.

We wish to thank Dr. T. Masuda for a gift of 6,7-dimethyl-8-ribityllumazine, Prof. J. de Ley for a culture of *Acetobacter rancens* 23, Dr. B. G. Audley for collaboration in certain experiments, the Agricultural Research Council for financial assistance (T. W. G.) and the University of Liverpool and University of Wales for personal grants (A. A. H.). The sonic disintegrator used was purchased with a grant from the Royal Society. Part of this work was carried out in the Biochemistry Department, University of Liverpool.

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ESSENTIAL OIL FROM FRESH *CANNABIS SATIVA* AND ITS USE IN IDENTIFICATION

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THE two main natural narcotics found in the illicit international traffic are opium and *Cannabis*. Considerable research has been done to develop methods of identifying and estimating opium constituents, and correlating these results to determine the country of origin[1–3]. A similar collaborative research programme has been inaugurated under the auspices of the Commission on Narcotic Drugs of the Economic and Social Council of the United Nations at Geneva, as an aid to tighten international *Cannabis* control measures[4]. Present methods of identification of *Cannabis* are based on botanical features, and colour and ultra-violet absorption tests on petroleum ether extracts of the plant or its resin. Since the tests are not satisfactory for forensic purposes, investigation of methods of *Cannabis* identification were begun by Farmilo *et al.*[5,6].

On the basis of previous experience in this laboratory[7,8], essential oils have been shown to be useful in plant characterization and origin determination. For this work thirteen samples of male and female fresh *Cannabis sativa* L. (the samples were identified

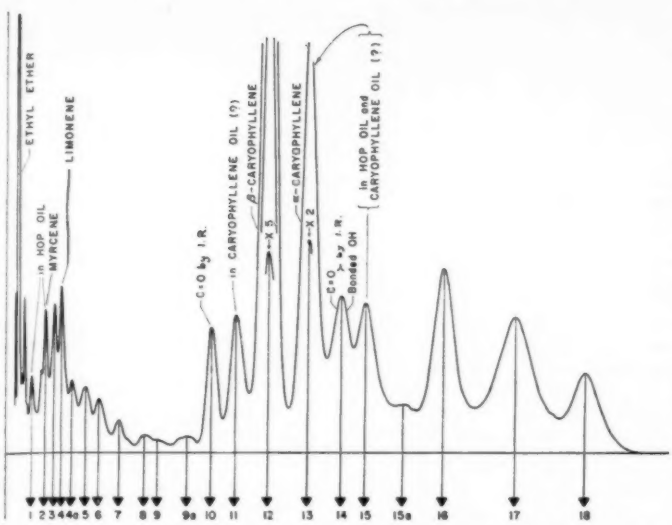

Fig. 1. Gas chromatogram. Oil from fresh large leaves of female *Cannabis sativa* (L.) Source: wild growth, Eastview, Ontario

botanically by Dr. D. Frankton, head of the Taxonomy Group, Weed Investigation Section, Plant Research Institute, Research Branch, Department of Agriculture; specimens are stored for future reference in the Arboretum of the Central Experimental Farm, Ottawa) found growing wild in ten different locations of the Canadian Provinces of Ontario and Quebec were used. (The samples of fresh *Cannabis* were provided through co-operation of the Narcotic Control Division, and the Royal Canadian Mounted Police, to whom we are indebted for this help.) The plants were stripped of leaves and inflorescences and the parts analysed separately. In addition, samples of dried *Cannabis*, hashish and charas of several different origins were studied. Hop oil and caryophyllene oil (courteously and generously supplied for this work by Fritzsche Bros., Inc., New York: the caryophyllene is produced from *Balsam copaiba* by washing and by fractionation) were used as references for characterization purposes.

Steam distillation of fresh male and female *Cannabis* plants at various stages of maturity yielded 0·05–0·11 per cent of oil (female and more mature specimens show highest yields). These results compare well with previous findings[8]. Gas chromatographic analysis of the oil (5 μl.) was carried out using a helium carrier gas-flow (75 ml./min.) through a column (2·5 m.) packed with 'Apiezon *M*' grease (20 per cent) supported on 'Chromasorb *W*' heated to approximately 220° C. More than thirty samples of oils have been assayed in this manner.

On one large oil sample, fractions corresponding to the constituents separated during gas chromatography were collected in small glass tubes packed loosely with glass wool, which were fitted on the exit end of the gas-chromatographic column. These fractions were dissolved in carbon tetrachloride and infra-red spectra were obtained using a Perkin Elmer Model 21, equipped with a scale expansion device and sodium chloride cavity cells (30 μl. capacity). After evaporation of the carbon tetrachloride solutions, the residues were redissolved in ethanol for application of colour and ultra-violet spectral tests. The spectra were taken in cells of 1 ml. capacity and 1 cm. path-length using a Beckman *DK*2 ratio recording spectrophotometer. The spectra of the neutral, acidic and basic ethanolic solutions were obtained. The colour tests, alkaline beam (ethanolic potassium hydroxide solution), Duquenois (vanillin and acetaldehyde in ethanol plus hydrochloric acid) and the Ghamrawy (*p*-dimethylaminobenzaldehyde in concentrated sulphuric acid) were carried out on a few drops of the ethanolic solution of each fraction.

The results of the above experiments are illustrated by the data given in Table 1 and a typical gas chromatogram is shown in Fig. 1. Table 1 lists the relative retention-time values (RRT-values) and the relative percentage composition of four oil samples obtained from leaves and inflorescences of fresh immature male and female plants picked in Eastview, Ontario. The illustration shows the gas chromatogram of the oil obtained from the large leaves of the female plant described.

A detailed examination of the chromatograms from the thirty *Cannabis* oil samples has shown remarkable consistency and reproducibility in the RRT-values of all peaks of the constituents 1–18. In comparing the RRT-values of one chromatogram with those of another a difference of 5 per cent in the values is taken arbitrarily as indicating a new component. Small peaks, such as those indicated by the numbers 4*a*, 9, 9*a*, and 15*a* are not always significant peaks in other chromatograms, and may vary in height and area depending on the part of the plant from which the oil is taken, for example, leaves, tip leaves, flowers, seed pods, etc. The roots and stems of the plants did not yield oil. The peaks 1–5 and those from 10 to 18 are thought to be most useful for the characterization of the oil of *Cannabis*.

Table 1. Results of Assay by Gas Chromatography of Oil from Fresh Immature *Cannabis* from Eastview, Ontario

Peak and fraction numbers	Relative retention times				Percentages			
	Leaves		Inflorescences		Leaves		Inflorescences	
	♂	♀	♂	♀	♂	♀	♂	♀
1	0·112	0·111	0·110	0·105	0·9	0·5	0·6	1·4
2	0·170	0·168	0·167	0·162	1·1	0·8	6·0	3·2
3	0·200	0·202	0·203	0·197	1·8	0·9	3·9	1·6
4	0·231	0·228	0·230	0·222	1·3	1·3	3·5	1·7
4*a*	0·269	0·265	0·266	0·258	0·5	1·6	1·3	0·6
5	0·323	0·319	0·307	0·300	0·9	1·1	1·6	0·5
6	0·375	0·369	0·366	0·357	1·2	0·8	1·4	0·5
7	0·448	0·443	0·435	0·435	•	0·5	•	0·4
8	0·555	0·540	0·535	0·530	0·7	0·2	•	0·3
9	0·590	0·586	0·586	0·575	•	•	0·4	•
9*a*	0·714	0·700	0·710	0·710	0·5	•	•	0·6
10	0·790	0·786	0·785	0·780	4·4	2·6	3·5	3·8
11	0·880	0·880	0·877	0·871	5·4	4·3	4·3	3·9
12	1·000	1·000	1·000	1·000	26·3	29·9	27·5	35·6
13	1·15	1·15	1·15	1·15	12·8	12·7	13·9	14·9
14	1·28	1·27	1·27	1·28	9·5	7·8	9·4	9·5
15	1·37	1·37	1·37	1·37	8·0	8·1	7·3	7·4
15*a*	1·51	1·51	1·50	1·50	•	•	•	•
16	1·66	1·66	1·65	1·67	9·0	9·4	4·5	4·5
17	1·93	1·92	1·93	1·97	10·7	11·8	5·2	5·1
18	2·20	2·12	2·20	2·29	5·0	5·7	5·7	4·6

• Peaks present, but too small to be measured.

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The essential oil of *Cannabis* resin (called either hashish or charas, if the source is either Eygpt or India respectively) has long been known to contain terpenes and sesquiterpenes which boil at temperatures much lower than the physiologically active narcotic constituents, tetrahydrocannabinols. The complexity of the composition of this oil has never been realized before, nor would it have been practical to assay it without the aid of gas chromatography. Simonsen and Todd[11] state that the essential oil from hashish consists mainly of *p*-cymene, 1-methyl-4-*iso*propenylbenzene and humulene (α-caryophyllene). They were not able to identify the presence of myrcene in hashish, although Wood, Spivey and Easterfield[12] had much earlier found it in charas from *Cannabis indica* (syn. *sativa*) obtained from Etawah in the province of Uttar Pradesh in India. By comparison of the RRT-values from the gas chromatograms of the oil of hops, another member of the family Moraceae, and of caryophyllene oil, peaks 2 and 4 are identified as myrcene and limonene respectively. β- and α-caryophyllene are found to be peaks 12 and 13. A number of other bands, 1, 2, 11 and 15, may be present (the same RRT-values) in the hop oil chromatograms and should be relatively easily identified for this reason. Infra-red spectral data indicate that the other compounds of terpenoid character show distinct functional groups, for example, hydrogen-bonded OH and C = O vibrations are evident. Others have aromatic characteristics which are revealed by their infra-red and ultra-violet spectra. More rigorous characterization of the remaining unidentified bands is now being carried out.

Chromatograms of oils from dried *Cannabis*, hashish and charas show distinct differences from those of the fresh oils.

The alkaline-beam colour test is stated to be positive when cannabidiol is present[13] and is given by some of the crude oils prior to fractionation on the gas chromatograph. No positive beam-test was obtained with any of the fractions isolated by means of gas chromatography. The Duquenois test is given by both α- and β-caryophyllene fractions; however, the former may react more strongly with this reagent. The question of concentration differences remains to be settled. The Ghamrawy reagent appears to be the least selective in its reactivity, the orange-red colour being given by fractions 10, 11, 15, 16, 17, 18. After dilution with water the colours in these tests changed to the characteristic blue colour. When applied to the original petroleum ether extracts of the plants of *Cannabis*, the alkaline-beam test is not always positive. The Duquenois and Ghamrawy tests have always been positive. It is therefore quite possible that the latter indicate the presence of the two caryophyllenes.

In conclusion, we wish to state that the fourteen peaks which are constantly present in the gas chromatograms of the oil serve to characterize, beyond a reasonable doubt, fresh, green *Cannabis sativa* L. found in Quebec and Ontario. Dried *Cannabis*, charas and hashish may also be distinguished and characterized on this basis. There are further useful chemical differences which help to identify them.

A full report of this work will be published elsewhere.

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INDUCTION OF MUTATIONS BY DEOXYRIBONUCLEIC ACID IN *DROSOPHILA MELANOGASTER*

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THE genetic activity of deoxyribonucleic acid (DNA) was conclusively demonstrated by the phenomenon of bacterial transformation[1,2], mainly in the genera *Diplococcus* and *Haemophilus*. When a genetically marked strain was exposed to DNA extracted from another with different genes, a new combination occurred in the recipient through the incorporation of one or more of the donor loci. A parallel phenomenon is that of transduction, which was demonstrated in *Salmonella*[3,4], where heritable changes of bacterial characteristics were brought about by bacteriophage-carried material assumed to be DNA. The consequences of administration of DNA in higher organisms has, so far, been a subject of conflicting reports. A phenomenon akin to bacterial transformation was reported in ducks by the Benoit group[5–7], but subsequent experimentation[7,8] failed to confirm these results. In mammals efforts to induce 'somatic transformation' have all failed[9,10].

For the investigation of the genetic consequences of DNA administration, *Drosophila* is a favourable test system. Techniques are available for the detection of mutations as well as induced transformation at specific loci. Both these aspects have been studied and the results are presented in this communication. While no specific gene replacement (locus transformation) was observed, the mutation-rate (especially as regards *Minutes*) was raised significantly above the control-level.

The DNA utilized in the present experiments was prepared in the Chester Beatty Research Institute; the first sample was extracted by Brookes from adults of the wild type strain (Oregon-*K*) of *Drosophila melanogaster*, according to Kirby's *p*-aminosalicylate/phenol method[11]. The DNA yield was extremely low and although free from RNA and protein, was in admixture with some unidentified impurities, probably polysaccharides. It was thought possible that the impurities might have been derived from the adult's chitinous exoskeleton, and accordingly attempts were made to extract DNA from embryonic tissue, namely, developing eggs. A method was devised for the production of eggs *en masse*, which enabled the collection of 2–3 gm. of pre-larval embryos per week, from a population of about 50,000 flies. The extraction of DNA from embryos was undertaken by Kirby by a modification of his previously published method[11]. This involved the breaking down of the tissues with diethyldithiocarbamate and phenolphthalein diphosphate before the application of phenol[12]. The percentage base ratio for a typical sample of the DNA separated by this method[12] was: guanine 23·8, adenine 29·3, cytosine 18·5, thymine 28·4. A sample of heterologous DNA from rat liver was also tested. This was prepared by the *p*-aminosalicylate/phenol method and gave nearly the same percentage base ratio[13] as the *Drosophila* sample (guanine 21·3, adenine 29·1, cytosine 20·1, thymine 29·5).

A cell-free extract of embryonic tissue was also tested for mutagenicity. This was prepared as follows: 1 gm. of eggs (pre-larval embryos) were mixed with 0·4 gm. of solid sodium chloride, and thoroughly ground in a glass homogenizer surrounded by solid carbon dioxide. The tissue homogenate was then diluted with 100 ml. of distilled water (to make the salt concentration isotonic) and filtered through glass wool. The filtrate was examined microscopically and proved to be a suspension free from whole cells and intact chromosomes. The concentrated salt treatment used probably disrupted the DNA/histone linkages, so that the above suspension might be looked upon as a dilute solution of DNA in admixture with other cell constituents.

The various samples of DNA were dissolved in isotonic saline (0·4 per cent sodium chloride) at the desired concentration (0·1–1 per cent) and the solutions were injected into the hæmocœl by our micro-injection technique[14]. For the mutagenicity tests wild-type (Oregon-*K*) males were treated and their progeny was scored for mutations. Chromosome fragments and visible mutations (autosomal dominants and sex-linked recessives) were tested for by the attached-*X* technique, and the sex-linked recessive lethals and visibles by the Muller-5 method[15]. In the transformation experiments, multiple recessive stocks were used carrying either 3 or 7 markers. Flies from these stocks were injected with a DNA solution extracted from the wild type, which would be derived from the full gene complement, including the normal alleles of the mutant markers. If any marked locus were substituted by the normal allele from the administered DNA, the phenotypic expression, corresponding to the substituted (transformed) locus, would be expected to revert to the wild type.

In both the mutagenicity and transformation experiments the progeny of the treated males was fractionated into 3-day broods by our standard technique[15]. Breeding was undertaken in mass-cultures with roughly equal male to female ratio.

The most convincing mutagenic effect of DNA treatment was the considerable increase in the rate of *Minutes* above the control-level (Table 1), which proved to be very highly significant in all experiments (Table 3). These mutations are a large class of non-allelic genotypic changes of comparable phenotype, expressed as various degrees of reduction in the length and thickness of the thoracic macrochætes. In extreme cases, the bristle effect is accompanied by various anomalies of the eyes as well as the wings (especially as regards venation). *Minute* mutants have a longer period of development, and are accordingly late in eclosion. They are dominant and lethal in the homozygous condition and are frequently demonstrable as deficiencies or small deletions in the salivary-gland chromosomes[16]. At the highest injected dose (1 per cent homologous sample, Table 1) the induced rate of *Minutes* was higher than the controls by a factor of nearly 12, while at the lower doses (with both the homologous and heterologous samples) the increase was by a factor of 2–3. The data from the homologous samples suggested that there might be a direct dose-effect relationship (Tables 1 and 3). In contrast to the situation with the *Minutes*, other induced F_1 autosomal dominants were rare, and were not significantly above the control-level; they were, accordingly, excluded from the present analysis.

The yield of the sex-linked recessive visibles in the treated series was also raised above the control-level both in the F_1 and F_2 experiments (Tables 1 and 2). For a statistical assessment of this trend, a certain amount of pooling of the data was undertaken to overcome the extreme rarity of visible mutations. The normal and mutant chromosomes in the F_1 and F_2 experiments for the control and treated series (from the same DNA sample and dose) were summed and compared in 2 × 2 contingency tables, and the results are given in Table 3. This analysis established the statistical significance of the increase in the visible rate with the high-dose experiments. The visibles from all experiments were subjected to allelism tests. The specific loci so far identified are listed here in their chromosomal order.

Control: *yellow* (*y*), *white* (*w*), *ommatoreductum* (*omm*), *dusky* (*dy*), *rudimentary* (*r*), *forked* (*f*).
Homologous DNA: *yellow* (*y*), *broad* (*br*), *stubs* (*sbs*), *pebbled* (*peb*), *tenuous-bristles* (*tnb*), *vesiculated* (*vs*), *smaller* (*sma*)-2, *vermilion* (*v*), *costakink* (*csk*), *miniature* (*m*), *narrow-scoop* (*nrs*), *fused* (*fu*).
Heterologous DNA: *brachymacrochaete* (*brc*), *white* (*w*), *rugose* (*rg*), *carmine* (*cm*), *copper* (*cop*), *rudimentary* (*r*)-2, *forked* (*f*)-2, *bronzy* (*bz*).

It would seem probable, therefore, that DNA treatment simply increased the incidence of mutation at the more responsive loci which occasionally mutated spontaneously. One point of interest, however, is that the *broad* (*br*), *rudimentary* (*r*), and *bronzy* (*bz*) mutants proved to be of the non-complementing[17] type, suggesting that the damage extended over all the cistrons of these complex loci. This may be an indication of the large size of genic damage by DNA, which of course is clearly suggested by the induction of *Minutes*.

The administration of DNA does not seem to result in a dramatic increase in the rate of the sex-linked recessive lethals (Table 2). Nevertheless, in one of the high-dose experiments (1 per cent DNA from adults, Table 3) there was a significant increase in the rate of these mutations above the control-level. It would seem probable, therefore, that larger scale experiments might permit a conclusive demonstration of the induction of recessive lethals by DNA.

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Table 1. The *Minutes* and Sex-linked Recessive Visibles (r) induced by DNA, Detected in the F_1 Males from Attached-*X* Experiments; Mosaic Mutants in Brackets

Broods	Control				Homologous DNA								Heterologous DNA			
					0·2 per cent (embryos)				1 per cent (embryos)				0·1 per cent			
		Minutes		r	*Minutes*			r	*Minutes*			r	*Minutes*			r
	Males	No.	Per 10^3	No.	Males	No.	Per 10^3	No.	Males	No.	Per 10^3	No.	Males	No.	Per 10^3	No.
I	9,619	8	0·83	3 (1)	9,638	19 (3)	1·97	1	1,993	20	10·04	2 (2)	11,682	36 (4)	3·08	2
II	9,538	12 (1)	1·26	3 (1)	4,931	8 (2)	1·62	1	1,349	27 (1)	20·01	0	7,899	15 (3)	1·90	1
III	9,669	8 (1)	0·83	1	7,653	32 (2)	4·18	3 (2)	525	8	15·24	0	7,916	9 (1)	1·14	2 (1)
IV	10,074	10 (3)	0·99	0	4,345	7 (2)	1·61	3 (2)	839	2 (1)	2·38	2 (2)	6,885	17 (2)	2·47	3 (2)
V	7,338	5 (1)	0·68	0	1,987	4 (1)	2·01	0	521	2	3·84	0	7,379	11 (1)	1·49	3 (2)
VI+	28,176	28 (4)	0·99	4 (1)	1,782	5	2·81	1 (1)	—	—	—	—	7,172	8 (1)	1·12	2
Total	74,414	71 (10)	0·95	11 (3)	30,336	75 (10)	2·47	9 (5)	5,227	59 (2)	11·29	4 (4)	48,933	96 (12)	1·96	13 (5)

Table 2. The Sex-linked Recessive Lethals (l) and Visibles (r) induced by DNA, Detected in the F_2 Progeny from Muller-5 Experiments; Mosaic Mutants in Brackets

Brood	Control			Homologous DNA						Heterologous DNA		
				1 per cent (adults)			1 per cent (embryos)			0·1 per cent		
	Chrm.	l	r	Chrm.	l	r	Chrm.	l	r	Chrm.	l	r
I	7,960	21	1	195	1	2 (1)	495	2	1	514	2 (1)	0
II	4,737	5	1	114	1	1	514	3 (1)	0	523	1	0
III	1,545	0	0	531	1	1 (1)	486	0	1 (1)	515	1	0
IV	1,078	2	0	573	4 (2)	1	485	0	1	486	1	0
V	487	0	0	88	1	0	2	0	0	473	1	0
VI+	—	—	—	122	0	0	141	0	0	1,394	1	1
Total	15,807	28	2	1,623	8 (2)	5 (2)	2,123	5 (1)	3 (1)	3,905	7 (1)	1
Mutants per 10^3	—	1·77	0·13	—	4·93	3·08	—	2·36	1·41	—	1·79	0·26

Table 3. Mutation-rates induced by DNA, and their Statistical Comparison with the Control Level

Experiment	Autosomal *Minutes*			*X*-recessive visibles			*X*-recessive lethals		
	Rate per 10^3	χ^2	*P*	Rate per 10^3	χ^2	*P*	Rate per 10^3	χ^2	*P*
Control	0·95	—	—	0·14	—	—	1·77	—	—
Homologous DNA									
1 per cent (embryos)	11·29	320·1	$\ll 10^{-9}$	0·95	21·7	10^{-6}	2·36	0·4	0·5
1 per cent (adults)	—	—	—	3·08	70·2	$< 10^{-9}$	4·93	7·1	< 0·01
0·2 per cent (embryos)	2·47	35·7	$< 10^{-8}$	0·30	2·9	0·1	—	—	—
Heterologous DNA									
0·1 per cent (rat liver)	1·96	22·2	$< 10^{-5}$	0·26	2·6	0·1	1·79	0·001	0·9

Mutagenesis studies of the fractionated progeny of males treated with various alkylating agents enabled the assessment of the time of sampling of the various stages of the testis in the brood-technique used in the present experiments. The post-spermatogonial stages (sperm, spermatids and spermatocytes) are predominantly utilized in the first four broods 0–12 days after treatment, whereas the spermatogonia are mainly recovered in the fifth and later broods (cf. ref. 18). In the light of the above conclusion, it is possible to infer the stages of spermatogenesis most susceptible to mutation by DNA. In practically all the experiments and with all the mutations scored (especially the *Minutes*), the maximal mutation-rate occurred in the first or second brood. There was one exceptional experiment (0·2 per cent homologous sample, Table 1) where the maximal rate (as regards *Minutes*) occurred in the third brood; but in no instance did the peak of mutability occur beyond the fourth brood. It is therefore certain that maximal mutagenicity by DNA occurs in mature sperm or late spermatids and minimal effect is on the earlier sperm mother cells, especially the spermatogonia. It should be pointed out, however, that even spermatogonia are not completely refractory to DNA; the rate of *Minutes* in the late progeny (fifth brood onwards) proved to be significantly greater than that in corresponding progeny fractions among the controls.

The DNA samples used in the above mutagenicity tests were extracted by procedures involving the utilization of compounds (such as phenol) which may not be mutagenically inert. Although it is virtually certain that none of these reagents was left in the purified DNA samples, it was felt desirable to ascertain the mutagenicity of DNA in the absence of such reagents. A cell-free tissue extract (in which the DNA/protein linkages were disrupted by concentrated salt) was accordingly subjected to an attached-*X* test. The injected suspension proved to be extremely toxic to the treated males, but it was possible to recover a single brood, containing 4,299 F_1 patriclinous males. In this sample 11 *Minutes* were scored—a rate of 2·55 per thousand, which proved to be significantly higher than the control-level ($\chi^2_{(1)} = 10{\cdot}0$; $P = 0{\cdot}001$).

The mutation-rate (as regards *Minutes*) induced by the tissue extract (1 gm. embryonic tissue in 100 ml. saline) was of the same order of magnitude as that induced by 0·2 per cent solution of pure DNA extracted from developing eggs (Table 1). If the mutagenic activity were entirely due to the DNA content of the tissue extract, the biological results would require the presence of 200 mgm. of DNA per gram of embryonic tissue; whereas in fact the extraction yield in Kirby's experiments was approximately 2–3 mgm. per gram of developing eggs[12]. This

might be an indication that the mutagenic efficiency of 'native' DNA was partially impaired by the phenol extraction procedure. Alternatively, it could suggest that the cell extract contained ingredients (such as nucleohistones or RNA) which contributed to the induction of *Minutes*. This problem is now under investigation.

The experimental details of the attempts at transformation of specific loci are given in Table 4. A multiple recessive stock was injected with DNA extracted from the wild type and the F_1 progeny was scored for reversion (transformation to the wild-type phenotype) at the various loci in the stock. In one experiment (1 per cent DNA from adults, Table 4) three autosomal markers were used: *brown* (*bw*: II), *ebony* (*e*: III), *poliert* (*pol*: IV), and only the male parents were injected with the DNA solution. In later experiments a stock with seven markers was used; X-chromosome with four mutations—*yellow* (*y*), *vermilion* (*v*), *miniature* (*m*), *forked* (*f*)—was incorporated in the gene complement together with the above three autosomals. A high concentration of DNA (1 per cent solution from embryos, Table 4) was injected either into both parents or into the male parent only. When injection was restricted to the fathers, only the number of the F_1 daughters was taken to represent the frequency of the potential transformants at the sex-linked loci. This precaution was taken in case a close association between the DNA and the treated chromosome is a pre-requisite of transformation. The high DNA concentrations had strong toxic and sterilizing effects on the treated flies, which prevented the recovery of large progeny. To overcome this difficulty, a low DNA dose was used and only the males were injected (0·2 per cent solution from embryos, Table 4) and this permitted a much larger test.

Table 4. NUMBER OF F_1 PROGENY OBSERVED FOR TRANSFORMATION AT SPECIFIC LOCI, AFTER TREATMENT WITH DNA FROM THE WILD TYPE

Sex injected	Females and males	Males	
Loci tested	*y v m f bw e pol*	*y v m f*	*bw e pol*
DNA sample			
1 per cent (adults)	—	—	1,185
1 per cent (embryos)	3,397	1,596	2,954
1 per cent (embryos)	99	757	1,547
0·2 per cent (embryos)	—	32,182	63,053
Total	3,496	34,535	68,739

As can be seen from Table 4, an overall total of 72,235 individuals was examined for transformation at the three autosomal loci, and 38,031 at the four sex-linked genes, but none was transformed to the normal allele. Any further increase in the size of sample would probably have involved the occurrence of spontaneous back mutation, which of course would not have been distinguishable from transformation. The present data, therefore, could reasonably be considered as strong negative evidence against the occurrence of specific locus transformation in *Drosophila*.

The exact mechanism whereby the injected DNA produces small chromosome deletions (*Minutes*) is difficult to interpret. Direct action through the induction of chromosome breaks as occurs with radiation can be safely excluded. The experiments were designed to reveal any viable chromosome fragments induced, but none was actually observed (in a sample of 83,893 scored females), indicating that the DNA treatment does not disrupt the polynucleotide chain of the chromosome. With the highly reactive alkylating agents, it was possible to show[19] that the induction of small deficiencies was not always correlated with chromosome breakage, but could also result from gene inactivation *in situ*. With DNA, however, it is virtually certain that neither the molecules themselves nor their degradation products could possess any alkylating ability to induce direct damage in the hereditary nucleoproteins.

It would seem, therefore, that mutations induced by DNA are the result of an indirect mechanism involving errors in chromosome replication, either during germ-cell multiplication or subsequent to fertilization during cleavage. Mitoses in the germ line do not appear to influence the mutation yield, since the effect of DNA treatment was higher on sperm and spermatids than on proliferating spermatogonia. However, this could be due to secondary factors such as a lower initial uptake of DNA, or a more rapid rate of its degradation by the cytoplasmic DNase in spermatogonia. It is reasonable to assume, therefore, that DNA incorporated in the germ cells at the time of treatment (especially in mature sperm), is passively transferred in association with the male chromosome complement to the zygote, and mutant induction actually occurs during chromosome replication at the first cleavage. An important feature of the phenomenon is that it appears to be non-specific: both as regards the inducing DNA (whether homologous or heterologous) and the induced mutations (predominantly non-allelic *Minutes*). The induction process, therefore, does not necessitate a specific exchange (akin to crossing-over) between the exogenous DNA and a particular locus on the chromosome, as was postulated[2] for bacterial transformation.

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PROPERTIES OF ISOLATED PLANT PROTOPLASTS

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LIBERATED bacterial[1] and fungal[2] protoplasts have proved to be of unique value in morphological, biochemical and genetic work on these micro-organisms. Following the recent demonstration in this laboratory[3] that plant protoplasts can be liberated from root cells of *Lycopersicum esculentum* after digestion of the cell walls by the enzyme cellulase, investigations into the properties of these liberated plant protoplasts have now indicated their unique potentiality for similar studies.

Protoplasts were liberated from root tips of tomato seedlings (*Lycopersicum esculentum* Mill. var. Sutton's Best of All) by treatment with *Myrothecium* cellulase[3]. Seedlings were grown under aseptic conditions and 3-mm. root tips from roots 20 mm. in length were used. The root tips were incubated at 27° C. for 2 hr. with freshly prepared *Myrothecium* cellulase in a 0·6 *M* sucrose modified White's culture medium[4] at *p*H 4·5. After treatment, the root tips were washed free of cellulase and mounted in special micro-culture chambers[5] in which liberated protoplasts could be examined microscopically, while freely suspended in the culture medium.

In the first experiment (exp. 1) root tips treated with cellulase were mounted in 0·6 *M* sucrose White's medium and microscopic measurements made of changes with time of protoplast and vacuole diameters. Most of the liberated vacuolated protoplasts were stable for about 12 hr. in this medium at room temperature; however, a few (approximately 1 per cent) burst after about 1 hr. Changes in protoplast and vacuole diameters of one of these rapidly bursting vacuolated protoplasts are shown in Table 1. The densely protoplasmic protoplasts which showed little evidence of vacuolation were stable over a period of several days, when maintained in a similar medium. During this time, they showed a slight tendency to shrink; they became opaque and the outer membrane became more clearly defined.

In exp. 2, 3-mm. root tips of tomato seedlings grown and treated with cellulase under similar conditions to exp. 1 were mounted in micro-culture chambers containing a 0·6 *M* sucrose White's medium at *p*H 4·5 with 10^{-5}, 10^{-7}, 10^{-9} and 10^{-11} gm./ml. of 3-indolylacetic acid (IAA, Kodak). There was a

Table 1. Changes in Protoplast and Vacuole Diameters of a Rapidly Bursting Vacuolated Protoplast

(0·6 *M* sucrose White's medium *p*H 4·5)

Time (min.)	Protoplast diameter in two directions at right angles (μ)		Vacuole diameter in two directions at right angles (μ)	
10*	32·8	33·6	29·3	29·3
20	33·6	32·8	29·3	29·3
30	34·5	34·5	30·2	30·2
40	34·5	34·9	31·8	31·8
60	37·3	37·3	36·3	30·7
80	38·0	38·0	37·3	31·1
92	Burst		25·9	27·6
96			24·1	24·1
98			24·1	24·1
101			24·1	24·1
104			24·1	24·1

* Timed from the initial washing to remove excess cellulase.

Table 2. Changes in Protoplast and Vacuole Diameters of a Vacuolated Protoplast

(0·6 *M* sucrose White's medium *p*H 4·5 with 10^{-5} gm. 3-indolylacetic acid/ml.)

Time (min.)	Protoplast diameters in two directions at right angles (μ)		Vacuole diameters in two directions at right angles: Vacuole *A* (μ)		Vacuole *B* (μ)		Vacuole *C*† (μ)	
2*	51·8	53·5	‡	‡	‡	‡		
4	55·2	54·5	34·5	34·5	‡	‡		
7	58·6	58·6	38·0	38·0	‡	‡		
8	58·9	58·9	‡	‡	12·1	12·1		
8½	Burst							
10			41·4	41·4	14·5	14·5	34·5	34·5
12			36·2	36·2	17·3	17·3	38·0	38·0
24			36·2	36·2	17·3	17·3	38·0	38·0

* Timed from the initial washing to remove excess cellulase.
† Gel-like, mobile vacuole. Measurements on this vacuole were only possible after bursting of the protoplast.
‡ No measurements taken.

very rapid bursting of vacuolated protoplasts. The most rapid and extensive bursting took place at 10^{-11} gm. IAA/ml. of 0·6 *M* sucrose White's medium and within 1 min. of treatment no vacuolated protoplasts or vacuoles could be observed. The least rapid bursting took place with 10^{-5} gm. IAA/ml. and changes in protoplast and vacuole diameters of one of these vacuolated protoplasts are shown in Table 2. There was not only a very rapid increase in the size of existing vacuoles present in the protoplasts, but also formation of new vacuoles both in already vacuolated protoplasts and in densely protoplasmic protoplasts. At an early stage in their development these vacuoles were more gel-like in nature and less rounded than existing vacuoles. When these vacuoles were liberated at this early stage of development, it was noted that the tonoplast was less clearly defined than in previously isolated more mature vacuoles. No inclusions were present in these vacuoles induced by IAA. Figs. 1 and 2 show typical changes in vacuolar form induced by 10^{-5} gm. IAA/ml. over a period of 3 hr. at room temperature. Photomicrographs were taken using a Leica camera and attachment mounted on a Baker series 4 microscope with a fluorite (× 100) phase-contrast oil immersion objective and 'Trilux' condenser. More rapid changes in vacuolar form were induced by lower concentrations of IAA, but photography of these was more difficult, due to the fact that marked changes took place during the required exposure time of 25 sec. A typical gel-like, mobile vacuole is shown in Fig. 1 (*A*); its formation was associated with active movement of the protoplasm and its associated granules.

Further cultural studies of protoplasts were carried out using specially prepared tomato root extract. This extract was prepared by homogenizing twenty 20-mm. roots in an all-glass homogenizer under aseptic conditions with 0·5 ml. 0·6 *M* sucrose White's medium at *p*H 4·5. Liberated protoplasts were cultured in this whole homogenate in the special micro-culture chambers previously described. Chambers were sealed with heavy mineral oil (paraffin liq-

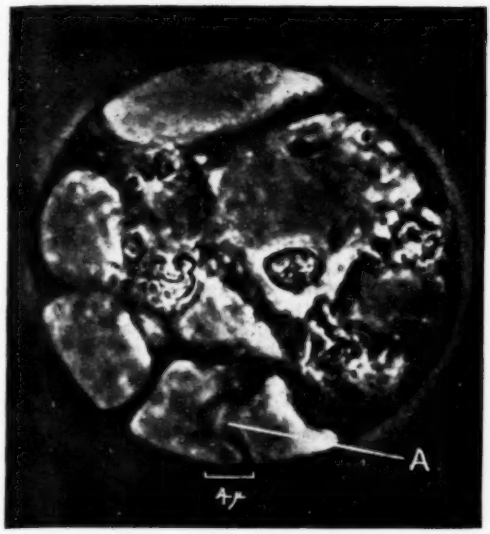

Fig. 1. Vacuolated protoplast (phase contrast). Treated with 10^{-3} gm. 3-indolylacetic acid/ml., 0·6 *M* sucrose White's medium for 15 min.

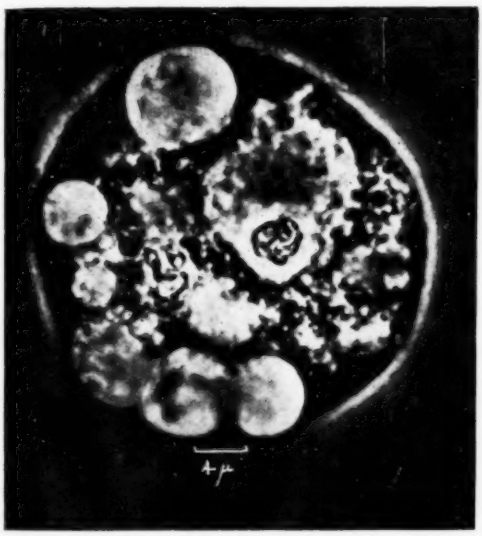

Fig. 2. Vacuolated protoplast (phase contrast). Treated with 10^{-3} gm. 3-indolylacetic acid/ml., 0·6 *M* sucrose White's medium for 3 hr. 15 min.

B.P. (Boots) to United States Pharmacopœia requirements), and under these conditions protoplasts could be maintained under aseptic conditions for at least one week (exp. 3). After culture for one day at room temperature, there was evidence of a more definite membrane around the more densely protoplasmic protoplasts. Furthermore, it was observed that several of these protoplasts had produced a bud-like protrusion (Fig. 3) after culture for two days in this tomato root extract; these protoplasts had by this time become highly opaque with particulate material massed near the surface of the now clearly defined outer membrane. These budding, densely protoplasmic structures closely resemble the budding cells observed[6] among single cells cultured in the presence of coconut milk. The implications of this finding will be discussed later.

It is known that mannitol[7] only very slowly penetrates into plant cells. Experiments were therefore carried out using mannitol as the osmotic stabilizer to determine whether the changes in opacity and membrane characteristics of these protoplasts observed in exps. 1 and 3 were being brought about by a toxic action of sucrose at the high molarity (0·6 *M*) necessarily used to maintain osmotic equilibrium in the culture medium. Protoplasts were cultured in 0·06 *M* sucrose, 0·54 *M* mannitol White's medium at *p*H 4·5. Changes in the opacity and membrane characteristics were observed similar to those in exps. 1 and 3. When comparable densely protoplasmic protoplasts were maintained in 0·6 *M* mannitol White's medium at *p*H 4·5 in the absence of sucrose, no such changes, however, could be observed. It would therefore seem highly probable that these changes are specific and are associated with sucrose metabolism and possibly cellulose synthesis.

These results indicate clearly the potentiality of the study of plant protoplasts in elucidating many of the present problems associated with growth and differentiation in plants. Owing to the absence of the cell wall, the response to nutrients and growth substances known to effect the growth and development of the whole root may be investigated at the protoplast-level with a greater attention to cytological detail and behaviour than has hitherto been possible with living cells. Moreover, the absence of the cell wall enables a more direct analysis of the mechanisms underlying these responses to be made. The protoplasmic effects of 3-indolylacetic acid and their possible association with increased water uptake by plant cells have recently been emphasized[8]. Other workers have focused their attention mainly on changes in the plasticity of the cell wall and possible chelation action of 3-indolylacetic acid[9–12]. Important as these effects on the cell wall may be, it is clearly possible to relate any effects of 3-indolylacetic acid observed with these protoplasts entirely to protoplasmic effects, and to effects on the plasmalemma and tonoplast. In view of the viscosity changes induced in vacuoles by 3-indolylacetic acid and observed in the present work, and the marked protoplasmic effects, it would seem that the application of simple osmotic relationships to explain the behaviour of these protoplasts in terms of vacuolar swelling may not be directly

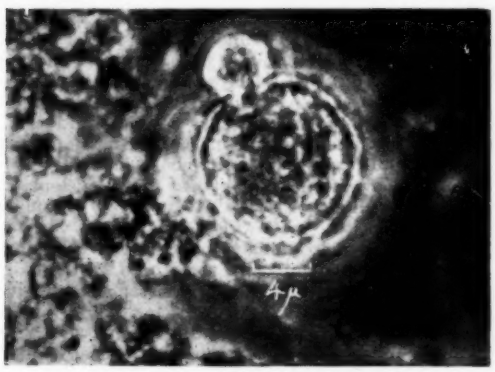

Fig. 3. Budding 'protoplast' (phase contrast)

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possible. The study of the osmotic behaviour of mechanically isolated clearly defined vacuoles[13] may yield little information concerning the actual osmotic relationships of vacuoles *in vivo* as they are induced and develop under the action of 3-indolylacetic acid and other similar growth-substances.

Budding of both highly vacuolated[6] and densely protoplasmic[14] cultured single cells has been reported. It has also been established that these 'buds' can become independently growing cells either separating from, or remaining attached to, the 'mother cell'[6] provided that this cell is highly vacuolated. In the case of budding densely protoplasmic cells, division of the 'mother' cell nucleus has been observed, resulting in the formation of a binucleate cell but the 'buds' in this instance do not seem to have a separate existence from the 'mother cell'. It is of some significance that densely protoplasmic structures with buds have been seen to be formed from densely protoplasmic protoplasts, when these were cultured in a White's medium tomato root extract, which probably contains many of the growth-substances associated with the White's medium supplemented with whole coconut milk as used in the culture of budding cells[6,14]. It seems likely that these protoplasts first develop some form of primary cellulose wall and then, parallel to their later development, they acquire some aspects of the behaviour of freely suspended isolated cells[6]. Clearly, further work with coconut milk and culture media supplemented with mannitol will be required before more definite answers can be given to the many questions arising from these new findings.

McQuillen[15] has noted that the optical density and dry weight of *Bacillus megaterium* protoplasts increases during incubation in suitable media, but that reversion to a bacillary form rarely, if ever, occurs. It is suggested that reversion only occurs when a trace of original wall material is still present, which can act as a primer or 'starter' for synthesis of new cell-wall material. These densely protoplasmic plant protoplasts are known to arise from meristematic cells in which the cell wall is very intimately associated with the protoplasm[3], and it may be of some significance that signs of reversion to a cellular form mostly occur among protoplasts such as these.

During the course of the present investigations, it was repeatedly possible to observe separated vacuoles, nuclei and masses of granular protoplasmic material following osmotic bursting of protoplasts. The development of osmotic bursting as a mild procedure for the separation and ultimate fractionation of protoplasmic components has been rendered difficult by the instability of these components arising as a result of the osmotic changes. The demonstration in the present work of the bursting ability of 3-indolylacetic acid is being utilized for the separation of protoplasmic components, since it is now possible to induce bursting of protoplasts in 0·6 *M* sucrose White's medium without changing the osmotic environment.

Thanks are due to Miss O. Wright for expert technical assistance. The award of a grant-in-aid from the Royal Society and a research grant from the Department of Scientific and Industrial Research is gratefully acknowledged.

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TUBER BULKING IN THE POTATO CROP

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YIELD in the potato crop is the resultant of the rate and duration of tuber bulking, the latter being a function of the time of tuber initiation and persistence of foliage. There is little precise information concerning the relative importance of these factors in accounting for differences in yield between varieties and different husbandry practices, and results obtained from field experiments at Sutton Bonington in the 1960 season may therefore be of interest.

Fig. 1 shows the course of leaf area development and tuber bulking, as revealed by weekly sampling, in Arran Pilot (the most widely grown early variety), Majestic (the most widely grown main crop) and Ulster Torch (a main crop, sometimes grown as an early, which is notable for its resistance to blight), planted on April 13.

The curve for increase in tuber weight with time, though similar in form, is displaced in time relative to that obtained by Borah and Milthorpe[1], and conflicts with the view that tuber initiation occurs near the time of maximum leaf area, and that the

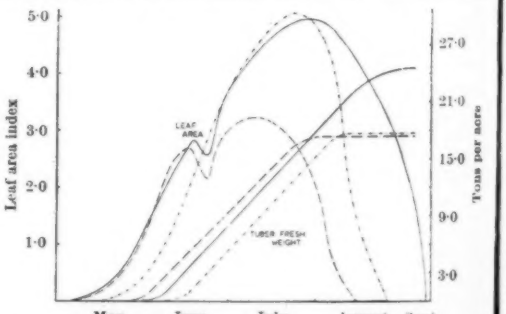

Fig. 1. Leaf area index and tuber growth in three varieties. ——, Ulster Torch; ·····, Majestic; – –, Arran Pilot

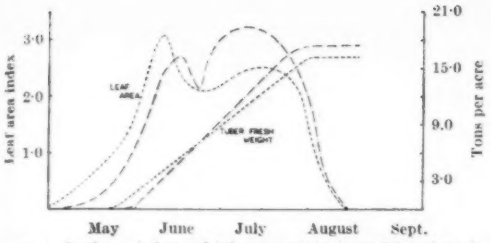

Fig. 2. Leaf area index and tuber growth in Arran Pilot planted on two different dates
– – –, Planted March 28; —·—, planted April 13

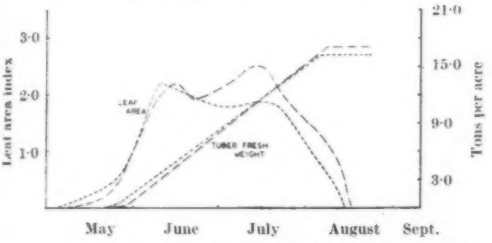

Fig. 3. Leaf area index and tuber growth in Ulster Chieftain planted on two different dates
– – –, Planted March 28; –·–, planted April 13

growth of tubers occurs at a time when leaf area is decreasing. In each variety the major part of tuber growth is made before maximum leaf area; indeed, decline in leaf area by senescence (as in Arran Pilot) or by blight (as in Majestic, and later Ulster Torch) is followed in about 14 days by the cessation of tuber bulking. The same general picture emerges with other varieties included in these experiments (Figs. 2 and 3). It should be noted, too, that though departures from linearity during the main period of bulking are generally small ($r = 0{\cdot}98$), they may be considerable when growing conditions are adverse: in the very dry 1959 season, particularly where inter-plant competition was intense, as with closely spaced plants, there was a mid-summer decline in leaf area and an almost complete cessation of tuber bulking. Subsequent recovery in leaf growth was associated with an increase in tuber weight.

Consideration of Fig. 1 suggests that tuber bulking-rate (here considered to be the slope of the curve during the linear phase) is, within fairly wide limits, independent of leaf area. On the other hand, Figs. 2 and 3 indicate that there may be a relationship between the two parameters, in so far as higher bulking-rate is associated with greater leaf area. The apparent discrepancy may possibly be due to leaf development in Ulster Torch and Majestic having surpassed the 'ceiling' at which crop growth-rate declines because of mutual shading of leaves. The pattern of total dry matter production in Arran Pilot and Ulster Torch is consistent with this hypothesis: there was no difference in this respect between the two varieties below leaf area index of 3, and little difference when the leaf area index of Ulster Torch increased to 5 while that of Arran Pilot remained at 3. This would suggest that there is no intrinsic difference between the varieties with regard to net assimilation-rate, but that the rate of Ulster Torch later declined relative to that of Arran Pilot because of the greater degree of mutual shading of leaves in the former variety.

Regarding the relative importance of the rate and duration of bulking in accounting for yield differences, Fig. 1 illustrates the paramount importance of the latter with these particular varieties in the 1960 season. By virtue of tuber initiation two weeks earlier, Arran Pilot had a yield advantage over Majestic of 4·4–4·8 tons per acre during the linear phase of bulking, but its final yield was slightly less because of earlier leaf senescence. Similarly, Ulster Torch had an advantage of 2·7 tons per acre over Majestic until the beginning of August, and gained a further 4·3 tons per acre afterwards, because of its blight resistance, which resulted in persistence of foliage for some 2–3 weeks after Majestic had virtually no green tissue. In contrast, differences in bulking-rate would appear to be of minor importance: with the exception of Ulster Chieftain (an early), which had a significantly lower bulking-rate than all others, varieties differed little in bulking-rate when planted at a time regarded as optimal for main crops (April 13.)

While duration of bulking is of great importance in determining final yield, it seems that attempts to prolong this by inducing early initiation, as may be done in the field by early planting of an early variety, may be ineffectual because of the reduced leaf area duration—a result of lower maximum leaf area and earlier senescence—associated with this practice. Thus, early planting may not in fact prolong the duration of tuber bulking and may result in lower bulking-rates. Figs. 2 and 3 show the increase in tuber-weight for Arran Pilot and Ulster Chieftain planted on two different dates (March 22 and April 13) and it is seen that appreciable yield advantage for early planting is short-lived, particularly in Arran Pilot. Planting before mid-April is, therefore, unlikely to increase yield unless early lifting is intended.

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BIOCHEMICAL CHANGES IN LETHAL STAPHYLOCOCCAL INFECTIONS IN MICE

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QUESTIONS regarding the specific cause of death in staplylococcal infections remain unanswered. *Staphylococci* are known to excrete a variety of toxic substances; but it is still uncertain that these are the major factors causing death. It has also been suggested[1] that endotoxins may be important and their effects could include: (1) direct toxic action on a process essential to life; (2) indirect action by the combination of a staphylococcal toxin with a normal body component to form a lethal compound; (3)

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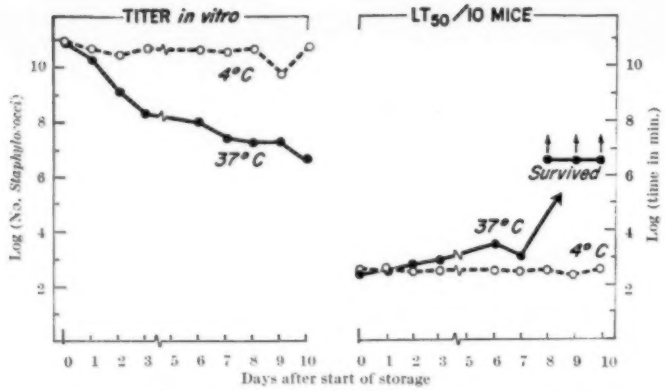

Fig. 1. Storage of staphylococcal culture at different temperatures

indirect action on the anabolism of a product essential to life and subsequent death due to deprivation; (4) the release, chemical cleavage or unmasking of a normal body component which, in excessive quantity, is toxic to metabolism.

The search for answers to this problem led to the following observations of the biochemical changes occurring in the blood of mice during a lethal staphylococcal infection.

We used the hæmolytic, coagulase-positive Smith strain of *Staphylococcus aureus*. This strain, originally phage-type 44*A*/42*E* (ref. 2), was isolated from a patient with osteomyelitis. Using the standard phage(s) it is, however, now non-typable. It has been maintained in our laboratory by regular *in vitro* transfer. Our investigations have shown a reasonably constant number of *Staphylococci* in the bodies of moribund mice regardless of the inoculation route. Therefore, this work was confined to the use of heavy-dose (0·1 c.c. = 10^9 organisms) intraperitoneal infections. The methods have been described previously[1]. The mice used were Carworth Farms (New City, New York) White Swiss males. The animals weighed 15–18 gm. and were fasted for 12 hr. before use.

Blood was collected from the mice by beheading with scissors. Within 1 hr. after collection both serum and heparinized blood samples were transferred to the biochemistry laboratories, where blood values were established.

The values 'at death' are the result of injections of 10^9 organisms into groups of ten mice. When two of the ten animals died the remaining eight were beheaded. This was felt to be a true value since it was found earlier that, with this inoculum and this route, the animals died in 4–5 hr., and all 10 animals usually died within a span of 20–45 min.

In previous work with this strain, a particular inoculum of *Staphylococci* had to contain $> 10^5$ viable organisms to be lethal. In the new experiments equal parts of a culture with high numbers of organisms were allowed to age at 37° C. and 4° C. Aliquots of these two samples were tested daily for their potency *in vitro* and *in vivo*. With the culture stored at 4° C., there was little change in the titre of the culture during the period of observation, and also little change in the time required for a standard injection of this culture to kill a group of mice. With the 37° C. culture there was a direct correlation between the decreasing titre *in vitro* and the gradually declining ability to kill mice (Fig. 1).

Biochemical values for normal and infected *CFW* mice are given in Table 1. A comparison of the values in the normal and intraperitoneally infected mice showed statistically significant differences in these four values ($P \leq 0{\cdot}05$): increased transaminase, alkaline phosphatase, and inorganic phosphorus and decreased sodium. There may be important changes in the cholesterol and serum carbon dioxide values, but results so far have not been significant. Abnormal biochemical values of the same order were obtained following infection by several other routes (Table 2).

To follow the trend of these biochemical changes, blood samples were collected at hourly intervals following infection and subjected to analysis. Fig. 2 shows the large increase in alkaline phosphatase and inorganic phosphorus during a 4–5-hr. infection. While there was no significant difference between normal and terminal values for fasting blood sugar, there was a distinct hypoglycæmia occurring between the first and second hours. However, due to homeostatic mechanisms, the sugar values at death were within our established normal limits for the mouse.

Table 1. Blood Biochemical Values for *CFW* Mice

	Normal	± S.D.	*S. aureus* at death*	± S.D.	*S. albus* at death
Fasting blood sugar mgm. per cent	52·7	12·20	35·8‡	14·50	56·0
Alkaline phosphatase s.u.	3·9	0·22	54·0†	24·40	4·8
Inorganic phosphorus mgm. per cent	9·7	0·71	12·8†	1·73	10·2
Transaminase s.f.u.	166·0	48·70	227·0†	64·30	299·0
Cholesterol mgm. per cent	139·0	33·80	106·0‡	28·30	181·0
Serum CO_2 m.equiv.	14·9	3·50	11·5‡	1·40	18·7
Sodium m.equiv.	149·8	3·10	145·1†	3·00	149·0
Potassium m.equiv.	8·9	1·27	8·8	1·07	
Chloride m.equiv.	110·5	5·40	109·6	2·40	
Blood urea nitrogen mgm. per cent	18·3	4·70	21·2	5·00	
Creatinine mgm. per cent	0·5	0·21	0·78	0·33	
Total serum protein gm. per cent	5·6	0·52	5·06	0·81	
Albumin gm. per cent	2·8	0·26	3·13	0·35	
Globulin gm. per cent	2·8	0·30	1·93	0·87	
Calcium mgm. per cent	11·1	1·72	10·8	1·27	
Uric acid mgm. per cent	2·8	0·61	2·7	0·58	
Acid phosphatase s.u.	1·6	0·45	1·97	0·22	
Total bilirubin mgm. per cent	0·0–0·2		0·1–0·3		
Cephalin flocculation	3+–4+		3+–4+		
Thymol turbidity u.	1·9	0·96	2·2	0·57	
Zinc turbidity u.	1·7	0·23	1·8	0·65	

* After 10^9 organisms intraperitoneally.
† Significant at $P \leq 0{\cdot}05$.
‡ Questionable significance (see text).
s.u., Sigma units; s.f.u., Sigma–Frankel units; u., Shank–Hoagland units.

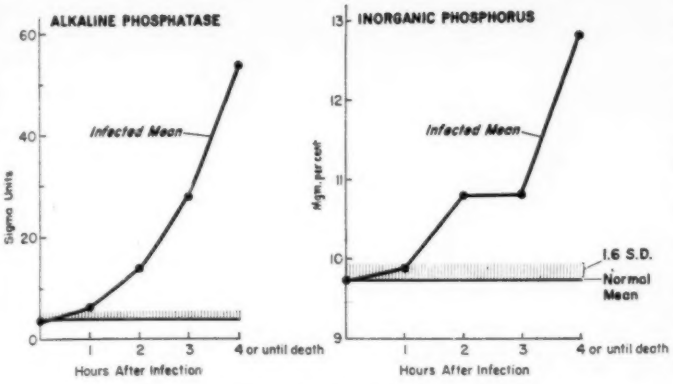

Fig. 2. Biochemistry of murine staphylococcal infections

The intraperitoneal injection of 10^{10} heat-killed *Staphylococci* produced early transient signs of illness, but no deaths, in the 14-day observation period. Blood samples collected at intervals of 8, 12, 24, 36 and 48 hr. and after 5 days showed no biochemical changes similar to those occurring during a lethal infection (also Table 2).

A strain of *S. albus* (*S. epidermidis* – coagulase-negative) obtained from a patient with septicæmia (case 6, ref. 3), although innocuous to mice in normal doses, was lethal after an inoculum of 10^{10} cells intraperitoneally. Blood values present at death failed to show the biochemical abnormalities produced by *S. aureus*. This was true with three other *S. albus* strains from patients with septicæmia.

Attempts to obtain a filtrable lethal factor from cultures of staphylococcal broth were unsuccessful. Intraperitoneal injections of 1·0 c.c. of sterile filtrate, obtained at various stages in the growth of the organism in brain–heart infusion broth were innocuous. Injections of 1·0 c.c. of sterile filtrate produced no deaths or obvious signs of illness in the animals, although it is possible that some toxic material was absorbed by the filter pad.

In addition, no filtrable lethal factor could be obtained from the serum of moribund animals when 0·5–2·0 c.c. were injected intraperitoneally into healthy recipient mice.

The results indicate that at the time of death from virulent *Staphylococci* in mice there is a high concentration of transaminase, alkaline phosphatase and inorganic phosphorus and a low sodium. A transient fall is noted in the blood glucose. These changes in the internal *milieu* are dependent on a heat-labile substance in the bacterial body. Blood serum with these biochemical changes, however, is not lethal intraperitoneally in an amount equal to an animal's circulating blood volume. *S. albus* does not produce these biochemical changes.

Various observations of changes in blood sugar, glycogen and phosphorus values occurring in experimental infections have been reported. Zeckwer and Goodell[4] studied anaphylaxis from killed bacteria injected into rabbits, and postulated that the hyperglycæmia present at death was the result of increased glycogenolysis rather than an interference with glucose metabolism. Injecting killed bacteria into rabbits, Delafield[5] showed that Gram-positive and Gram-negative organisms elicited different responses. Gram-negative bacteria caused an initial hyperglycæmia followed by a low content of sugar in the blood at 24 hr., and at the same time there were increased inorganic phosphorus values. Gram-positive bacteria, on the other hand, caused no significant changes in blood sugar or inorganic phosphorus. Kun and Miller[6], using *Salmonella* endotoxin in rabbits, reported essentially the same result, and in addition showed significantly reduced glycogen content of liver and muscle tissue. Similar blood sugar results were reported recently[7] following injections of anthrax toxin into rabbits. The hyperglycæmia was correlated with a decreased content of liver glycogen. A marked increase was also observed in the activity of alkaline phosphatase and transaminase.

An apyrase (adenylpyrophosphatase) can be extracted from several bacteria, but *Staphylococcus aureus* excretes apyrase into the culture media[8]. This enzyme liberated inorganic phosphorus from adenosine triphosphate, adenosine diphosphate, adenosine monophosphate and diphosphopyridine nucleotide. This work should be extended to *in vivo* tests.

We are studying at present the effects of accentuating or neutralizing these biochemical derangements in an attempt to devise a biochemical treatment for staphylococcal infections in the mouse[9]. This should

Table 2. Biochemical Values 'At Death'

Determinations	Control*	Live organisms					Dead organisms
		Intraperitoneal* (0·1 c.c.)	Intravenous (0·1 c.c.)	Intracranial (0·02 c.c.)	Intramuscular (0·5 c.c.)	Subcutaneous (0·3 c.c.)	Intraperitoneal† (1·0 c.c.)
Fasting blood sugar mgm. per cent	52·7	35·8	36·0	46·5	39·5	51·0	49·8
Alkaline phosphatase s.u.	3·9	54·0	27·9	11·0	33·0	6·8	1·6
Inorganic phosphorus mgm. per cent	9·7	12·8	14·0	12·0	13·9	13·4	10·2
Sodium m.equiv.	149·8	145·1	148·0	141·0	138·0	151·0	152·0
Transaminase s.f.u.	166·0	227·0	220·0	240·0	230·0	400·0	300·0
Cholesterol mgm. per cent	139·0	106·0	107·0	100·5	119·5	132·0	125·0
Serum carbon dioxide m.equiv.	14·9	11·5	21·0	21·6	11·1	19·3	21·4

* 5–6 determinations in these; 2–3 in the rest.
† Values present at peak of obvious illness (12 hr.).
Titre = 10^{10} organisms/c.c.

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also indicate if these biochemical changes were an integral part of the death process, causal factors, or non-essential correlates.

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ELECTRON MICROSCOPE OBSERVATIONS OF THE RECEPTORS IN THE SENSORY VESICLE OF THE ASCIDIAN TADPOLE

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THE free-swimming larvæ of sea squirts react to light and gravity in a manner that ensures their wide dispersal. The receptors sensitive to these stimuli are situated within the cerebral vesicle of the tadpole. In the species examined these consist of a unicellular otolith, and an ocellus made up from about ten cells. Since there are only about 60 cells in the whole of the cerebral vesicle the connectivity patterns of these receptors might yield important clues as to the manner in which they influence the motor systems of the tadpole.

Tadpole larvæ of *Ciona intestinalis*, *Phallusia mammillata* and *Ascidia nigra* were fixed in buffered osmic acid or potassium permanganate and embedded in 'Araldite'. Some specimens were stained in 1 per cent phosphotungstic acid in absolute acetone before embedding. The material was examined in a Siemens Elmiskop electron microscope.

The Otolith. This gravity receptor protrudes into the cavity of the vesicle from a small group of cells, which form a mound on the ventral wall (Fig. 1). The otolith itself is a single cell, consisting of an intracellular dense black mass of material, a narrow stalk, and an L-shaped 'foot'. The foot is situated in the group of cells forming the mound, whereas the narrow neck and dense body project into the cavity of the vesicle. This body is dense in the sense that it is opaque to light and electrons as well as being heavy. It is a sphere 15μ–25μ in diameter, and since its density is greater than that of the other cell contents it probably acts as the weight that distorts the otolith cell during rotation of the tadpole. The fact that it is densely black and is situated near the ocellus suggests that perhaps it may also function as a shutter, cutting off light from the ocellus intermittently as the otolith moves during rotation of the tadpole.

The remaining part of the otolith cell, which is free within the cavity of the cerebral vesicle, is conical in shape and contains the nucleus. The junction between fixed and free portions of the otolith cell is organized in a complex manner. The cell membrane is folded back on itself to form a collar around the stalk of the cell in such a way that the collar makes a socket around the stalk. The organization of this collar is further complicated because the cytoplasm is filled with distended vesicles of the endoplasmic reticulum. The collar is roughly cylindrical in outside shape, whereas the inner surface slopes inwards conically from a wide base above, to a narrow neck below. The stalk at the junction is only about 0·75μ in diameter. It seems remarkably thin to support the mass of the otolith. This is obviously the junction where maximum distortion will occur as the otolith moves in the cerebral vesicle and must therefore be suspected as the transducer region.

The foot region of the otolith cell, that is, the fixed region, extends at right angles to the long axis of the remainder of the cell, to give the whole structure an L shape. The right angle of the L is occupied by a further cell, which is probably a neurone. This cell differs from the other cells in the mound in that it is

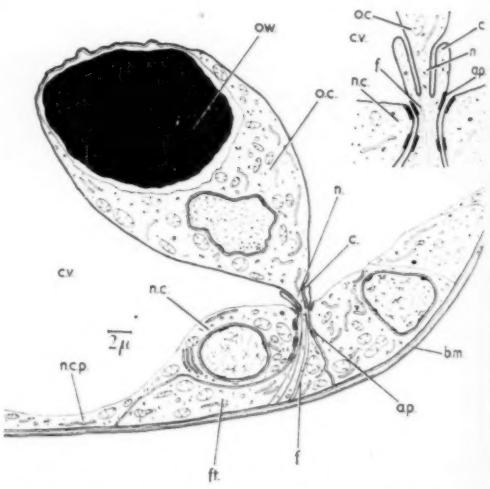

Fig. 1. Otolith (transverse section). Drawing from an electron-micrograph. The gravity receptor projects into the cavity of the cerebral vesicle, and is free to move within the cavity during rotation of the tadpole while swimming.
Inset, to show details of the neck region of the otolith cell. The vesicles of the endoplasmic reticulum have been omitted.
a.p., Attachment plaques; *b.m.*, basement membrane; *c*, collar of transducer; *C.V.*, cavity of the cerebral vesicle; *f.*, anchoring filaments; *ft.*, foot process of the otolith cell; *n.*, neck of the transducer region; *n.c.p.*, nerve cell process, probably an axon; *o.c.*, otolith cell body; *o.w.*, otolith weight

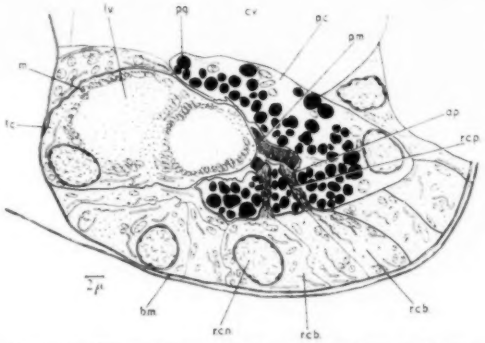

Fig. 2. Ocellus (transverse section). Drawing from an electron micrograph. The photoreceptor may be considered as made up of three parts, a lens system, a pigment cup, and a retina *a.p.*, Attachment plaques; *b.m.*, basement membrane; *C.V.*, cavity of the cerebral vesicle; *l.c.*, lens cell; *l.v.*, lens vesicle; *m.*, mitochondrion; *p.c.*, pigment cell; *p.g.*, pigment granule; *p.m.*, piled photoreceptor membrane; *r.c.b.*, retinal cell body; *r.c.n.*, retinal cell nucleus; *r.c.p.*, tubular process of the retinal cell

oval in shape and has a long cell process, which is probably an axon running initially transversely across the cerebral vesicle. The oval shape of this cell is such that the short arm of the L is pyramidal in shape whereas the long arm is narrow in the middle and wide at each end. The constant position and presence of this cell in the angle of the L, together with its process, suggest that it is probably the neurone that transmits the impulses generated by movements of the otolith. This may be effected by generator potentials in the junctional region causing action potentials to be generated in the neurone; or the neurone may be distorted mechanically by movements of the otolith.

Both sides of the vertical pyramidal region of the foot are attached to their surrounding cells by specializations of the cell membranes similar to the attachment plaques described by Odland[1], and probably serving to anchor the foot firmly to the surrounding cells.

There is a set of fibrils in the otolith running from the membrane folds of the fixed free junction through the cytoplasm of the foot region to the basement membrane. They arise from the narrowest innermost part of the neck region and are attached to the innermost membrane of the basement membrane. They are 150–250 Å. in diameter and perhaps serve as a mechanical anchor for the neck region so that it cannot be excessively distorted.

The Ocellus. The ocellus (Fig. 2), the photoreceptor, is made up of three parts, a pigment cup, a lens system and a retina. It is situated at the caudal end of the cerebral vesicle.

The pigment cup situated between the lens and the retinal cells is a single cell with the lens cell contained partly within its concavity. It contains a mass of pigment granules, varying in diameter between 0·5 and 2μ and probably all bound by a membrane. The granules are more concentrated in the thick central region than at the periphery of the cell. The nucleus is confined to the non-granular apex of the cell. The function of the pigment cell is obviously to keep stray light from falling on the photoreceptor endings, thus ensuring that only light that has been orientated by the lens falls on them.

The lens system is usually unicellular, but may consist of two or more cells. There are usually three lens vesicles, arranged in a line along the main axis of the concavity of the pigment cup. The lens vesicles vary in diameter between 6μ and 12μ. They are usually spherical in shape. Each vesicle is separated from the rest of the cytoplasm of the lens cell by a single-layered sphere of mitochondria. There is no membrane separating the lens vesicle contents from the rest of the cytoplasm of the lens cell. There are no cell organelles within the vesicles, which are filled with a granular material similar in composition to the background granular material of the extravesicular cytoplasm, but in a less-dense concentration. These granules, 150–200 Å. in diameter, are usually distributed homogeneously throughout the vesicle; however, occasionally they have a greater concentration at the centre of the vesicle than at the periphery. The nucleus of the lens cell is usually in that part of the cell distal from the pigment cup.

The retina consists of about eight cells arranged in a row outside the dorsal arm of the pigment cup. They are conical cells, resting on the basement membrane that separates the cerebral vesicle from the rest of the body. These cells narrow distally to form tubes that penetrate the pigment cup and end as piles of membranes closely applied to the lens cell in the base of the concavity of the pigment cup. The retinal cells can thus be considered to have three parts: the main cellular part outside the pigment cup, the tubular part, which penetrates the pigment-containing cell, and the piled membrane photoreceptor part.

The main cellular part is of a truncated cone shape, narrowing from a wide base resting on the basement membrane down to a narrow neck at the outer edge of the pigment cell. This part of the retinal cell contains the nucleus. As yet no synapse-bearing foot process has been found.

The single tubular part of each cell penetrates the pigment cell and is cylindrical, about 6μ long and 0·75μ in diameter. It extends from the apex of the retinal cell body to the piled membrane photoreceptor part of the cell. The tube contains numerous (50–100) filaments running along its long axis. These filaments are solid, some 150 Å. in diameter and without cross striations. They do not run parallel to one another but are tangled together along the length of the tube. These filaments do not penetrate into the retinal cell body cytoplasm. The retinal cell tubular part is separated from the cytoplasm of the pigment cup by its own cell membrane, and the cell membrane of the pigment cell. Along the lengths of these two membranes are membrane thickenings similar to attachment plaques. This tubular part probably corresponds to the connecting cilium described by De Robertis[2] in the retinal rods of the rabbit. It is unlike it in that in transverse section it does not contain tubules in the normal organization of the cilium (that is, nine peripheral and two central).

At their central ends each tube is continuous with a piled photoreceptor membrane. This corresponds well with the usual vertebrate rod pattern, consisting of piles of membranes arranged in disks 150–200 Å. thick and up to 2·5μ in diameter. The piles are several μ long and are arranged perpendicular to the direction of the light falling on them.

Thus the main morphological feature of vertebrate photoreceptors, a layered lamellar structure, is present in the sea-squirt tadpole.

I wish to thank Prof. J. Z. Young and Drs. E. G. Gray and J. D. Robertson for advice and criticism.

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LIMITATIONS OF GEOMETRICAL MODELS FOR COMPLEMENTATION MAPPING OF ALLELIC SERIES

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THE first attempt to construct a gene map from the functional relations between alleles was carried out in the scute series of *Drosophila melanogaster*[1-3]. This system of 'step-allelism' assumed, by analogy with 'deletion mapping', that the complementary relations expressed in the progeny derived from various allelic crosses represented spatially distinct sub-genes or regions of the scute gene. It was, however, based on the further assumption that each sub-gene controlled one specific character—in this case, a characteristic set of bristles. This latter assumption turned out to be erroneous because the map relations were no longer maintained in the same linear order when temperature differences[4] or genetic modifiers[5] were used in conjunction with the allelic crosses. Furthermore, a radiation breakage analysis[6] of this small region indicated discontinuous organization of the genetic units with many fewer elements than proposed in the step-allele analysis. Nevertheless, at least two separable elements (achæte and scute) were definitely established[7].

In recent years a similar use of functionally complementary relations among alleles has been made, and the process has been called complementation mapping[8,13]. In some systems the complementation units inferred from the phenotypically non-mutant heterozygotes are unambiguously distinct, spatially, as judged by recombination, as well as functionally. These systems require all the point mutations in one complementation unit to complement all the point mutations in the other complementation unit. Such a relation has been typically designated as non-allelism. In other cases, there are additional point mutations, not known to involve deficiencies or other re-arrangements of the genetic material, which cannot complement at all. Such mutants appear to extend their genetic effect to all the complementation units in an allelic series. This is the more typical expectation of complementation studies for allelic series, and it has been referred to as intralocus complementation. Further use of this technique has produced complementation maps involving four, six or more complementation units (Table 2). In all these cases one or more complementation units may be affected by a point mutation. This expression of effect by the mutant is known as its complementation pattern.

In evaluating these complementation maps, several assumptions and variables must be explored. One requirement is a knowledge of the number of complementation patterns which are consistent with linearity. A second factor would be the number of other possible geometrical models which could satisfy a complementation mapping procedure. A third important problem is whether the maps derived from complementation studies are co-linear with those derived by recombination analysis (either by the use of associated outside marker genes or by use of matrices ordered for a topological correspondence to linearity[9]). Each of these criteria involves difficult genetic problems which defy a simple analysis. For example, it is now widely recognized that almost all allelic series (especially in micro-organisms) are bound to yield recombination among most of the members tested if large enough numbers of progeny are scored[10]. This might create a misleading sense of confidence in the correspondence of a complementation map to the spatial separability demonstrated by recombination. Although the complementation test often assumes and predicts spatial separability, it must be kept in mind that allelism *per se*, in present-day genetic thinking, also assumes the likelihood of a separability, by recombination, of the mutationally altered sites in different alleles. Another difficulty not always appreciated is the non-randomness of the mutations occurring within a genetic region[11]. The resulting mutational spectrum for a given gene may be interpreted to have arisen as a consequence of hot spots, varying length of the genetic sub-units, or the sensitivity of certain regions to mutational damage because their alterations involve a region critical to the specification of the active functional region of the gene product. Other areas of the gene, altered with the same frequency, may produce fewer mutations because the final product of the gene is not altered in a region critical to its function. Such apparent intragenic mutational differences may lead to inaccurate interpretations of the number of complementation units in an allelic series.

Mathematically, the relations between the complementation units and the complementation patterns possible for allelic series are summarized in Table 1. These show that a linear map involves only a portion of the patterns possible in abstract terms, especially as the number of complementation units increases. Two other maps are possible by use of this process—circular and conical models. The special geometrical cases, such as triangularity for three complementation units, the tetrahedron for four units, and pyramids with polygonal bases for larger numbers of units, all have properties topologically identical to either the circular or conical models mentioned. Notice that if four or more complementation units are proposed for a gene, all its complementation patterns can be satisfied by the conical model. If only one or two complementation units are proposed, both linearity

Table 1. Relation between Complementation Units, Patterns and Maps

Complementation units proposed	Maximum patterns for linearity	Maximum patterns for circularity*	Maximum patterns possible†
1	1	1	1
2	3	3	3
3	6	7	7
4	10	13	15
5	15	21	31
6	21	31	63
7	28	43	127
–	–	–	–
–	–	–	–
–	–	–	–
n	$n\left(\frac{n+1}{2}\right)$	$n(n-1)+1$	2^n-1

* Without radii.
† For four or more complementation units the maximum pattern can be achieved by a cone with (n) faces or a circle with n radii.

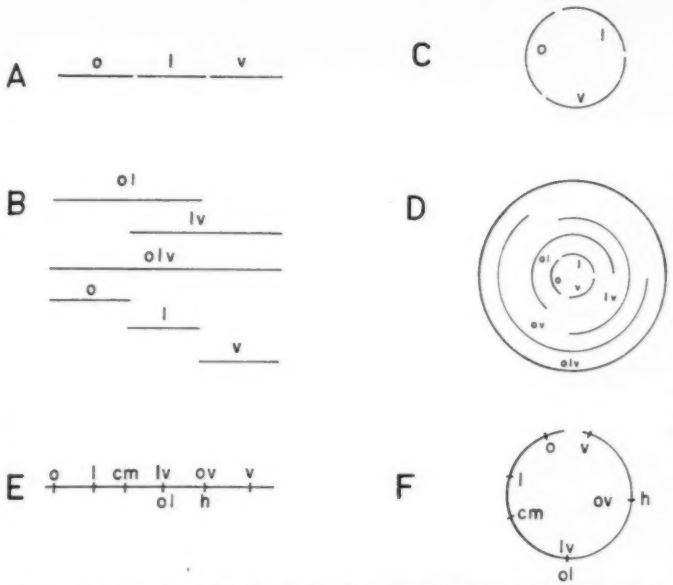

Fig. 1. *o*, Oblique wings; *l*, lethality; *v*, thoracic vortices; *cm*, comma, a weakly expressed mutant with *o* and *v* effects; *h*, humpy-like, an allele with exaggerated *o* and *v* effects. *A*, the linear complementation map with three complementation units. *B*, the six-complementation patterns consistent for linearity. In this sequence, *o–l–v*, the omitted member is *ov*. If the sequence were *l–o–v*, the omitted member would be *lv*; for *o–v–l*, the omitted member would be *ol*. *C*, the circular complementation map with three complementation units. *D*, the seven complementation patterns consistent for circularity. *E*, the recombination map—all sites recombine with the other neighbouring sites and order is determined by positions of outside marker genes in the recombinants. *F*, the recombination map, if visualized as a circular map for correspondence with complementation map *D*. Note the discontinuities in *o* and *l* effects in both map *E* and *F*

and circularity can be satisfied. It is, of course, unlikely that these non-linear maps have any physical correspondence to gene structure.

An example of non-linearity in complementation mapping can be found in the dumpy series in *D. melanogaster*[12]. Here the three effects which are expressed by either the wing (*o*), thorax (*v*) or viability (*l*) produce a maximum of $2^n - 1 = 7$ complementation patterns. A linear map can be satisfied if $n\left(\frac{n+1}{2}\right) = 6$ patterns are obtained. However, the known phenotypes of the point mutations in the dumpy series suggest seven patterns: *o*, *l*, *v*, *ol*, *lv*, *ov*, and *olv*. This would correspond to a circular complementation map, since $n(n-1)+1 = 7$. Essentially similar arguments were proposed earlier to reject an interpretation of this series as a nest of three closely linked genes[13]. Notice that when the various complementation maps are compared with the directly determined recombination map, there is no obvious relation (Fig. 1).

It is, of course, possible that developmental complexities in *Drosophila* make allelic complementation an analogous, unrelated process to that found in viruses, bacteria and fungi. Nevertheless, all the intensively studied allelic series in micro-organisms must meet certain requirements for a critical demonstration of a spatially linear complementation map of the gene. These are, first, that the maximum permissible number of complementation patterns for the set of complementation units has been achieved and not exceeded. Because of the non-randomness of the allelic series collected, there is no easy way to eliminate the possibility of complementation patterns greater than $n\left(\frac{n+1}{2}\right)$, but certainly several alleles should be assigned to each of the complementation patterns. Secondly, the map obtained by the complementation technique, if it is intended to reflect gene structure, should be co-linear with the recombination map[14]. To date, this has not been achieved in a clear-cut way since, as examination of Table 2 indicates, the necessary maximum has not been achieved or the status of linearity is not an exclusive one. In the absence of these rigid criteria, it may be misleading to state that two genes or alleles are separated by a distance of several complementation units. It is entirely possible that the complementation units may have no real physical significance[15] for gene structure, and thus their maintenance for complementation mapping would require such *ad hoc* hypotheses as spreading effects[16], polymerization of gene products[17], or cytoplasmic recombination at the protein or ribonucleic acid template-level[8]. Additionally, it must be borne in mind that not all complementation patterns may exist in an allelic series; that co-linearity may exist for only certain portions

Table 2. Status of Complementation Mapping in Various Loci

Locus	Symbol	Organism	Comp. units proposed	Comp. patterns obtained	Comp. patterns needed	Comp. alleles used	Modal No. per pattern	Consistency with linear map	Ref.
Histidinol dehydrogenase	his D	*S. typhimurium*	2	3	3	33	6	Yes*	16, 21
Glutamic acid dehydrogenase	am	*N. crassa*	3	6	6	11	1	Insuff.	22
Dumpy	dp	*D. melanogaster*	3	7	6	31	3	No	12
Histidine *E*	his *E*	*S. typhimurium*	4	8	10	11	1	Insuff.	16
Histidine *B*	his *B*	*S. typhimurium*	4	9‡	10	24	1	Insuff.	16
Pantothenic acid	pan-2	*N. crassa*	6	12	21	23	1	Insuff.	13
Adenylo-succinase	ad-4	*N. crassa*	7	16	28	51	1	Insuff.	24
Isoleucine-valine	iv	*N. crassa*	†	18	§	19	1	Insuff.§	23

* Also consistent with circular complementation map.
† Three 'regions' proposed.
‡ Three additional patterns are redundant.
§ Must be 6 or more complementation units, with 21 patterns minimal for consistency with linear map.

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of complementation and recombination maps; and that complementation may exist for two alleles which, by recombination analysis, are shown to occupy the same site.

Although it is not possible either to confirm or to invalidate the linearity of complementation mapping, the phenomenon of complementation is a real one and its value for understanding gene function, and indirectly, gene structure, is not denied[18]. Nor does the limitation imposed by the concept of the complementation unit rule out the cytoplasmic reassociation of component polypeptides of an enzyme or other protein[19,20]. Such demonstrated mechanisms of cytoplasmic complementation may be independent of the structural assumptions inherent in the complementation mapping of the particular gene involved. It seems, however, that the alterations in the gene molecule and the functional disturbances produced in its final product are more complex than can be accounted for on the assumption of mere co-linearity between genetic recombination and genetic complementation.

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RELATIONSHIP BETWEEN SIMPLE AND SERIAL REACTION TIMES

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IT is one of the oldest findings in experimental psychology that when a subject has to respond to a discrete signal some measurable delay will occur. But this delay is due to many variables which are not yet fully understood. Where a response is required to a second stimulus, closely following a first, the second reaction time (RT_2) has generally been shown to be much longer than the simple reaction time, so much so that a different kind of explanation has been proposed for the so-called 'psychological refractory phase'. It was the underlying hypothesis of these experiments that work on serial reaction times may have been handicapped by stemming primarily from Telford's[1] work and by ignoring the variations which occur in the simple reaction time itself. Delays in responding in the serial situation have been treated as if they were unique[2], but may in fact closely parallel fluctuations in the simple reaction time. This article reports some of the findings of an investigation designed to make an overall comparison of simple and serial reaction times.

Subjects sat in a sound-treated room wearing padded ear-phones and responded to an auditory signal by releasing a morse key. The design of the experiments, unfortunately complicated, manipulated fixed and variable intervals before both the first and second signals. Table 1 sets out these conditions. After a subject had pressed a 'ready' key there was a warning delay of either 1, 2, 3, or 4 sec. In the regular condition it was known which of these intervals would be used; in the irregular it might be any one of the four periods. The first click occurred at the end of the warning delay and was followed by a fore-period terminated by a second click. There were eight fore-periods ranging from 25 to 1,000 m.sec. and again these might be regular (a known interval) or irregular (any one of the eight). Catch trials were used with all regular procedures.

Data were taken from five practised subjects. In all some 20,000 readings were recorded, excluding practice data, and, with the use of an electronic computor, were examined by analysis of variance. Any differences which are discussed were statistically significant.

At procedures I and II simple reaction times were examined after regular and irregular warning delays. The mean RT's with the regular warning delay at the 1-, 2-, 3- and 4-sec. intervals were 134·3, 146·6, 146·3 and 152·6 m.sec., respectively. At the same intervals for the irregular procedure these means were 164·4, 146·4, 146·6 and 146·5, respectively. The 1-sec. warning delay produced the quickest response in the regular procedure and the slowest in the irregular.

To facilitate comparisons for the remaining procedures the results for all the fore-periods based on a mean of the four warning delays are presented in Table 1 and have been graphed in Fig. 1. The striking feature of the graph is the manner in which reaction time increases at the minimum fore-period with the increasing ambiguity of the experimental conditions. With procedure III, where both warning delay and fore-period were regular, the reaction time was almost the same for the fore-periods from 25

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Table 1

Procedure No.	Warning delay	Fore-period	Signal responded to	Fore-periods (m.sec.) 1 25	2 50	3 100	4 150	5 200	6 250	7 500	8 1.000
III	*R*	*R*	2	155	158	152	153	161	150	126	125
IV	*R*	*I*	2	175	166	154	155	156	159	147	131
V	*I*	*R*	2	189	176	159	154	151	144	123	115
VI	*I*	*I*	2	192	180	159	148	152	146	130	113
VII	*I*	*R*	1 and 2	244	234	199	185	173	163	126	108
VIII	*I*	*I*	1 and 2	265	246	218	195	197	180	136	119

Mean response times (m.sec.) to the second signal after a regular (*R*) or irregular (*I*) warning delay and fore-period. Warning delay intervals 1–4 sec. Fore-period, 25–1,000 msec.
Analysis of variance, using a random model as the conservative index of significance, gave *F*-ratios which were significant at the 0·01 level or above between the fore-period conditions of procedures V and VII, VI and VIII, and VII and VIII.

to 250 m.sec. For the irregular fore-period of procedure IV it increased slightly at the 25- and 50-m.sec. interval. With procedure V, where the 1–4 sec. warning delay was irregular, followed by the second click at a prescribed interval, subjects were slower in responding at the shorter fore-period than in procedure III where the warning delay preceding the first click had been regular. There was a further increase in reaction time at the shortest fore-periods for procedure VI, where both warning delay and fore-period were irregular. But it is obvious from the larger displacements for procedures VII and VIII that the delay resulting from perceiving an immediately preceding signal by no means accounts for all the interference of these responses. Procedures V and VI were identical with VII and VIII except that, in the two latter, subjects not only observed the first click but also manually responded to it. The slower reaction times which occurred to the second click as a result of this previous response were considerable and persistent. The results for procedures VII and VIII were not unlike those found by other work on the refractory phase, though the magnitude of the delay was not so great as in some earlier experiments.

If the results for procedures VII and VIII were considered in isolation, or against some so-called 'average' reaction time, it is apparent why an explanation of the 'psychological refractory phase' type would be necessary. There is a large increase as against some standard reaction time of say 140 m.sec. for an auditory stimulus. But in the present work the comparison is with reaction times produced under conditions which have varying degrees of similarity to those of the final procedures, VII and VIII. It is clear that with increasing uncertainty as to when either signal would occur there was an increasing reaction time at the shortest fore-periods. It is certainly the case that even the observation of a previous and irregular signal retarded the response to the critical signal, as in procedures V and VI. But this perception, contrary to the assertion of some workers, has been shown to be only one of many variables contributing to the delay. The manual response to the first click, in procedures VII and VIII, definitely increased further the delay in responding to the second click.

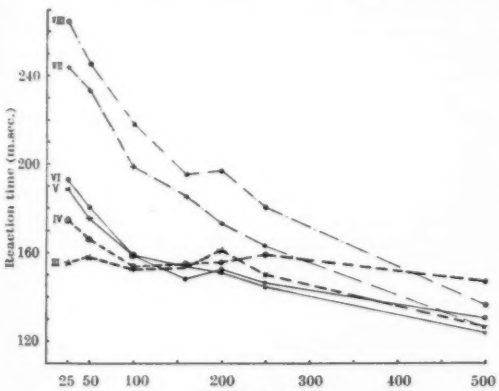

Fig. 1. Mean response times (m.sec.) to second signal at fore-periods 25–500 m.sec.
III: × – – ×, regular warning delay, regular fore-period.
IV: ○ – – ○, regular warning delay, irregular fore-period.
V: × — ×, irregular warning delay, regular fore-period.
VI: ○ — ○, irregular warning delay, irregular fore-period.
VII: × —●— ×, RT_2 irregular warning delay, regular fore-period.
VIII: ○—●—○, RT_2 irregular warning delay, irregular fore-period.

From this series of experiments it would seem that the variability in the simple reaction time, shown so clearly by Breitwieser[3] and Woodrow[4], is closely mirrored in the serial reaction-time situation. Theorists have been prone to postulate rigid fixed intervals of time between the completion of one response and the initiation of the next. The present work casts serious doubts on this approach. It would support previous work in so far as no two reaction times are overlapping, and there is the usual pronounced delay with the standard serial reaction-time situation of procedures VII and VIII. But this situation is only an extreme example of how RT_2 can be delayed. If we think of a minimal simple reaction time only occurring when the organism is optimally prepared to make a response to a signal, we have evidence from present and previous work how the reaction time will vary with uncertainty as to when the signal will occur. It would appear that in cases of such uncertainty the state of readiness of the organism cannot be raised to one critical peak point in time. In the case of the serial reaction time any signal which has to be perceived and which occurs immediately before the critical second signal, serves to lower the state of readiness of a subject. The disruption seems related to the degree of uncertainty of the first signal, and the extent of the response which is required. We might say that in the serial reaction-time conditions, the restriction where a subject has to observe a first signal and respond as quickly as possible to it is an optimally disturbing condition on the preparatory set for perceiving and responding to the second signal (RT_2). It would follow that one possible explanation for the delay in responding to the second signal is along lines identical with those for explaining the variability in the speed of responding to the first.

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LETTERS TO THE EDITORS

RADIOPHYSICS

Trans-Equatorial Backscatter Observations of Magnetically Controlled Ionization

A ROTATING backscatter equipment operating at Brisbane on a frequency of 16 Mc./sec. with a highly directional aerial[1] has been modified to record echoes with ranges up to 15,000 km. It is found that on 95 per cent of afternoons echoes are returned from a north-easterly direction with ranges of 6–12,000 km. Typical sequences are shown in Figs. 1 and 2, where the plot is one of range versus magnetic azimuth from Brisbane.

Fig. 1. Range-azimuth records of trans-equatorial backscatter. 6/10/60

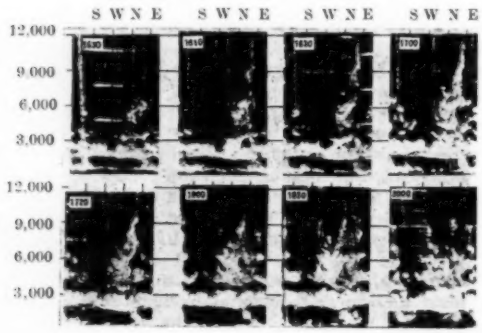

Fig. 2. Range-azimuth records of trans-equatorial backscatter. 2/1/61

It is well known that the electron density of the *F* region in the afternoon is less near the magnetic equator than in two sub-tropical belts at geomagnetic latitudes of about ± 20°, and several theories have been put forward to account for this phenomenon[2–5]. These theories involve the electrodynamic uplift of ionization at the equator and its subsequent downflow along the magnetic field lines to the sub-tropical regions near ± 20° M. In addition, Wright[6] has produced meridianal sections of the quiet day ionosphere, showing the extremely rapid variations of the ionization contours as a function of height and latitude.

If one compares the locations of the long-range echoes referred to here with the parallel of magnetic latitude 20° N. (that is, computed from the actual dip values), it is found that the echoes generally lie very close to this line. Two specific instances from the sequences of Figs. 1 and 2 are shown in Fig. 3.

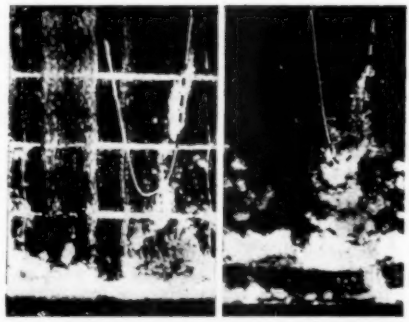

Fig. 3. Range-azimuth records for *a*, 1600/6/10/60 and *b*, 1700/2/1/61 with overlay plots of the 20° N. parallel of magnetic latitude

The question of the propagation mechanism is rather involved as the echoes come from regions in which the local time may be up to 6 hr. ahead of the local time of observation at Brisbane. It is believed, however, that the main mechanism is that of 'tilt-mode' propagation proposed by Villard *et al.*[7], sometimes with a single hop to the *F* region before the equatorial jump. This hypothesis is being examined further by computing the expected oblique profiles of ionization density at various times and in various directions from Brisbane.

The fact that the eastern limb of the echo is the predominant feature is probably due to the changing ionospheric conditions as one proceeds from east to west with changes in time of several hours. By the time the western limb should be visible at long ranges, the electron density south of the equator is too low for the necessary propagation conditions to be set up.

It appears probable that such observations of the whole length of the belt of high-density ionization are only possible with highly directive antennæ. The signal is often weak and scattered and with a broad beam antenna may well merge into the background noise. This point is to be checked shortly, and further tests will be made with a swept-frequency backscatter sounder operating at 15–30 Mc./sec. and with an additional fixed-frequency sounder operating at a higher frequency.

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RADIOASTRONOMY

Central Component of the Radio Source Centaurus *A*

THE identification of the radio source Centaurus *A* with the peculiar galaxy *NGC* 5128 was first suggested by Bolton, Stanley and Slee[1]. The radio source consists of an extended component with dimensions of about 2° × 4° and a compact central component. The extended source has recently been shown by Bolton and Clark[2] to consist of two components. The compact central component was investigated in 1953 by Mills[3]. His observations indicated that the radio emission was associated with the wide dust band of *NGC* 5128. Recent measurements by Twiss, Carter and Little[4] have shown that the central source also consists of two components. This result was also obtained by Moffet and Maltby[5]. In a more recent radio investigation of *NGC* 5128, reported in this communication, I find that the radio source is not associated with the wide dust band.

Using the variable spacing interferometer at the Owens Valley Radio Observatory, Centaurus *A* has been observed with different antenna spacings and in different orientations. The measurements were taken at wave-lengths of 21·6 cm. and 31·3 cm. In order to determine the position of the minor axis, the source was observed for about 2 hr. on either side of upper culmination with a north–south interferometer. At the latitude of this Observatory the source is close to the horizon, and a relatively long horizontal antenna separation is therefore needed to investigate the source in the north–south direction.

In Fig. 1 the observed fringe amplitude is plotted against hour angle for two different antenna separations. Fig. 1*a* shows the observations at 31·3 cm. using a horizontal antenna spacing of 800 ft., while the curve in Fig. 1*b* is obtained at 21·6 cm. with the antennas 1,600 ft. apart. The accuracy of the individual points in the curves is ± 3 per cent of the maximum intensity of the source. In the following, we will neglect any change in the brightness distribution between these two frequencies. Changes in the fringe amplitude in Fig. 1 are due to changes in the effective antenna separation as well as to changes in the orientation of the source in the fringe pattern. From Fig. 1 we find that one maximum occurs at an hour angle equal to 1h. 10m. in both curves. This means that the minor axis is in a position angle of about 135°. Using the maxima and the minima in Fig. 1, it was found that the major axis is in position angle 46·5° ± 2·0°. Although the position angle of the minor axis deduced by Mills[3] was 40°, his observations would not be inconsistent with the position angle of 136·5° reported here. Indeed, all Mills's[3] observations are in reasonable agreement with the interpretation given here, if we take into account the fact that the source consists of two components.

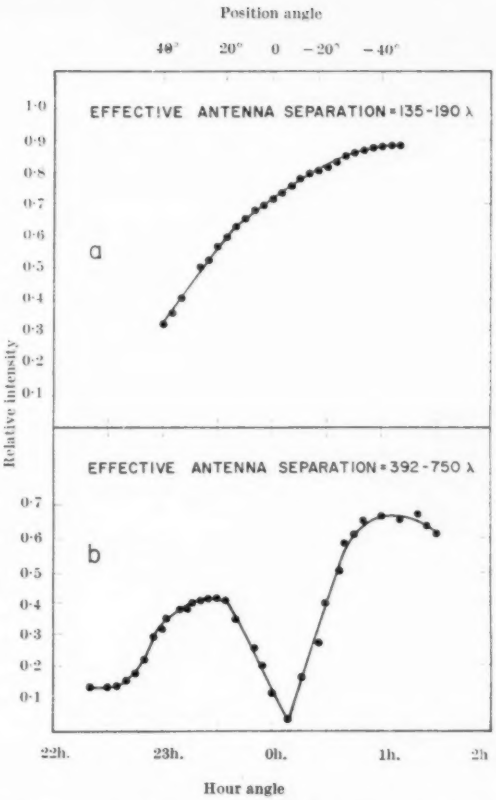

Fig. 1. Observed fringe amplitude plotted against hour angle. *a*, Observed curve at 31·3 cm. using a horizontal antenna spacing of 800 ft.; *b*, observed curve at 21·6 cm. with the antennas 1,600 ft. apart

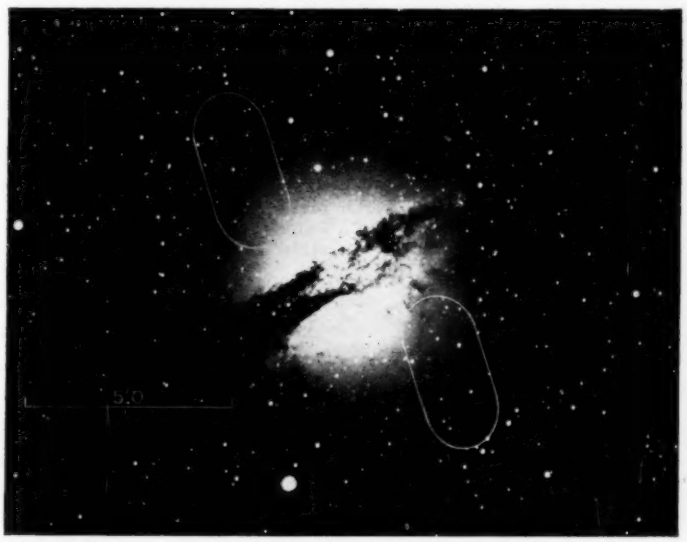

Fig. 2. Half-intensity contours of the two components in the central radio source of Centaurus *A*. The photograph of *NGC* 5128 is from the Mount Wilson and Palomar Observatories

Comparing the two maxima in Fig. 1*b*, I find that the maximum at 23h. 30m. is much lower. It may be shown that, if the source consisted of two circular components, the maximum at 23h. 30m. in Fig. 1*b* would have approximately the same value as the maximum at 1h. It is therefore reasonable to suggest that the components are elongated.

We are now in a position to derive a more detailed picture of the source. From the observations along an east–west base-line by Moffet[6], a diameter of about 2 min. of arc for each component is indicated. The curves in Fig. 1 were compared with those which would be produced by a model source consisting of two equal components 2·0′ in diameter, separated by 7·1′ along the major axis in position angle 46·5°. The elongation of the two components was found by comparing the observed and calculated curves. The interpretation was simplified by assuming that the two components have similar shapes. The shape of the components and their position is indicated in Fig. 2. A good fit to the observed curves was obtained. No significant deviation was present in Fig. 1*a*. In Fig. 1*b* the discrepancy between the calculated and the observed curve was found at the minima, where the calculated curve was equal to zero. Deviations between the curves were present between hour angles of 0 and 20m. and between 22h. 20m. and 22h. 50m. The deviation from the observed curve gave an upper limit of 1·3 to the ratio of the intensities of the components. This is in good agreement with Moffet's observations in the east–west direction[6].

From Fig. 2 it is evident that the radiation originates in a region outside the wide dust band. The major axis is approximately normal to the dust band. Each component is elongated approximately along the major axis of the extended source. It is interesting to note that the photographs of *NGC* 5128 in red light[7] show elongation of the galaxy along the same major axis as the central radio source.

It is significant to note that the sources lie on the polar axis of the galaxy. It is possible that the sources are associated with the escape of high-energy electrons from the galaxy through a polar fringing magnetic field. The non-existence of radio emission from the dust band may be explained as due to the presence of a high gas density in that region. Processes such as ionization loss will then dominate and the electrons will not lose energy by means of radio emission.

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PHYSICS

Experiments on the Compression of Plasma in Cusp Geometry

Containing plasma by cusp-shaped magnetic fields has for some time been considered a promising possibility because of the favourable stability properties predicted theoretically[1–4]. To investigate this configuration experimentally, an apparatus has been built in which shock pre-heated plasma is compressed by a rising cusped magnetic field.

The apparatus is shown in Fig. 1. The cylindrical 'Pyrex' discharge vessel is 20 cm. in diameter and 75 cm. long, and cone-shaped at each end to fit inside single-turn pre-heat coils. The two single-turn cusp field coils are of 22 cm. diameter with separation variable between 6 and 16 cm., and for the present experiments each coil was connected to a 600-μF. condenser bank rated at 40 kV. When charged to 24 kV., this provided an oscillating magnetic field of about 50 kilogauss at the line and point cusps, with a period of 50 μsec. The shock waves which ionize and pre-heat the initially neutral gas are produced by the simultaneous discharge of two auxiliary condenser banks into the pre-heat coils. The current in these coils rises to 200 k.amp. in a time of 1·5 μsec. Experiments have been carried out using hydrogen gas at pressures in the range 20–40 millitorr and argon and helium at somewhat lower pressures.

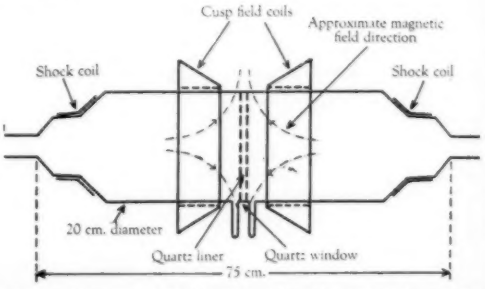

Fig. 1. Diagram of discharge vessel and associated magnetic field coils

With hydrogen at 22 millitorr, spectroscopic measurements on the bremsstrahlung and recombination continua (McWhirter, R. W. P., unpublished work) in the region of the Balmer series limit indicate that a plasma electron density of 2×10^{15} cm.$^{-3}$ and electron temperature of about 5×10^{4} °K. is produced by the shock pre-heating system.

The effect of the rising cusp field on the pre-heated plasma has been studied with a framing camera; examples of the observed phenomena are shown in Fig. 2. Fig. 2*a* shows the shocks approaching and meeting followed by the first half-cycle and part of the second half-cycle of the cusp field. Fig. 2*b* shows the growth of an instability after 2–3 μsec. in a typical case of the contraction velocity exceeding the estimated speed of sound. Fig. 2*c* shows an example of flute-type instability in helium in the very early stages of compression. Fig. 2*d* shows a condition in hydrogen in which no gross instability is observed and the full course of adiabatic compression can be seen.

It is suggested that the observed instabilities are of the Rayleigh–Taylor type[5], that is, resulting from the acceleration of the plasma by the magnetic field.

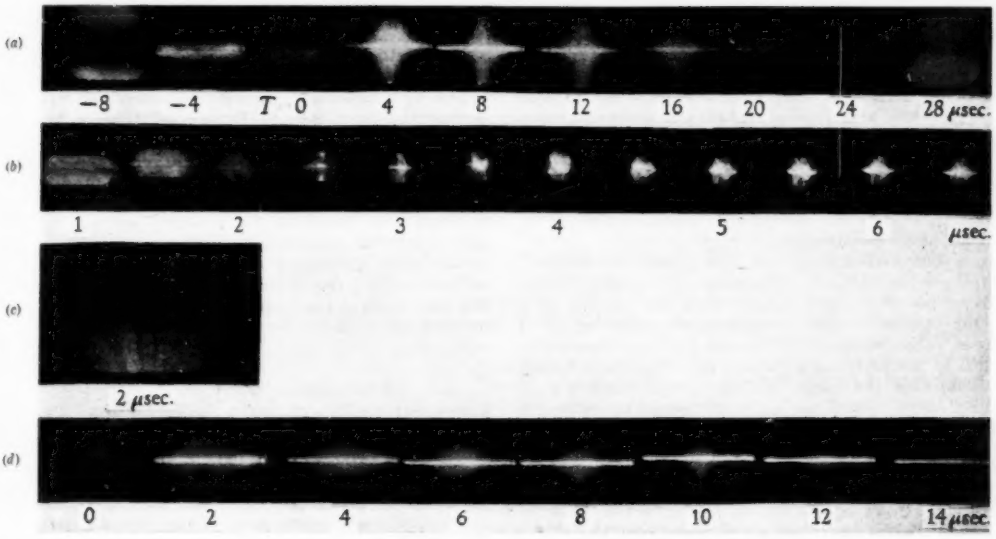

Fig. 2. Framing camera photographs of entire section of discharge tube between cusp field coils. The time at which the photographs are taken is marked. The cusp field starts rising at $T = 0$. (*a*) Hydrogen–argon (5 per cent) mixture; 20 millitorr; coil separation, 16 cm.; exposure, 2 μsec. per frame. (*b*) Argon, 5 millitorr; exposure, 0·25 μsec. (*c*) Helium, 10 millitorr; exposure, 1 μsec. (*d*) Hydrogen, 35 millitorr; exposure, 1 μsec.

Such acceleration occurs during the initial stages of the compression (Fig. 2*c*) and, additionally, on the reflexion of complex strong shock patterns at the boundary in those cases where the contraction velocity exceeds the speed of sound in the pre-heated plasma (Fig. 2*b*). The criterion for stability in the relevant case of a plasma accelerated by a curved magnetic field is[1]:

$$\frac{B^2}{4\pi R} > nMf$$

where R is the radius of curvature of the magnetic field lines (taken positive for lines convex to the plasma), f is the plasma acceleration (positive for acceleration directed from the plasma towards the magnetic field), and nM is the plasma density. The observations are qualitatively consistent with this criterion in that the system is more unstable in heavier gases, and there is evidence of considerable stabilization during the later stages of compression when the sign of f changes. It should be emphasized that the instabilities discussed here are associated with the establishment of an equilibrium state, and no comprehensive results concerning the stability of such an equilibrium have been obtained in these preliminary experiments.

However, when operating conditions are such that these initial instabilities are not present, the cusp boundary appears to remain quite sharp during the first quarter-cycle. Analysis of framing camera pictures for some of the most stable and reproducible conditions, namely, 22 millitorr of hydrogen, gives volume compressions of about 15–20 after 8 μsec. Spectroscopic measurements at this time indicate an increase in density greater than 10 and an increase in electron temperature from 5×10^4 °K. to a value greater than the upper limit of 2×10^5 °K. measurable by the method.

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A Proposal for a Tunable Source of Radiation for the Far Infra-red using Beats between Optical Masers

In this communication, the possibility is considered of generating radiation in the far infra-red by beats between optical masers.

Oscillations from optical masers have been detected at several different wave-lengths in the region of 7000 Å. using ruby[1,2], and also calcium fluoride doped with samarium. Calcium fluoride doped with uranium ions[3] has also been used to produce oscillations at 25,000 Å. So far these solid-state optical masers have only been operated in pulses, but seem to be potentially capable of continuous wave operation.

Recently, the dependence on temperature of the oscillation wave-length of a ruby optical maser has been investigated[4] and this has been found to vary from 6934 Å. at −180° C. to 6953 Å. at 210° C. If beats between a fixed frequency and a thermally tuned optical maser could be generated, then the difference signal could be tuned from centimetre to submillimetre wave-lengths. Indeed, there is no

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reason why the whole of the far infra-red spectral region could not be covered by selecting the frequency of oscillation of one optical maser and thermally tuning another. The spectral purity of the actual oscillation signal is determined chiefly by crystal imperfections, and typical values of oscillation line-width lie between 0·1 Å. and 0·02 Å. Thus a possible spectral width of about 1 Gc./sec. would be expected at the beat frequency, which would be narrow enough to be used as a tunable source of radiation in the far infra-red, even with the present state of the art of optical maser construction.

In a practical arrangement, one optical maser could be operated at, say, the temperature of liquid nitrogen, and its power output mixed with the output of a second optical maser temperature controlled to a pre-set value between 25° C. and 210° C. The wave-length of the beat signal would then be continuously variable over the range of 250μ–550μ. Such a wide tuning range (which undoubtedly could be extended by a larger variation of temperature or by using crystals with a larger temperature coefficient of oscillation wave-length) is unlikely to be equalled by a single maser operating directly in the far infra-red.

As the bursts of oscillation are only 0·5 msec. in duration, fairly exact synchronism of the oscillation bursts would be required if separate optical masers were to be used. However, two optical masers would not necessarily be required to produce oscillations at different wave-lengths, since experiments with concentrated ruby[2] have shown evidence of simultaneous oscillations at 7009 Å. and 7041 Å. Beats between these two signals would give an output signal of approximately 150μ wave-length. The problem of synchronism of oscillations would not arise provided that the energy from the ultra-violet flash-lamp is greater than the threshold-level necessary to maintain both oscillations simultaneously. Such an arrangement would be rather restricted in tuning range, since both oscillation wave-lengths would be shifted by approximately equal amounts on cooling or heating the crystal. On the other hand, this may be an advantage from the point of view of frequency stability of the beat signal.

Since the pulsed output of the optical maser is typically 10 kW., any system of optical mixing must be capable of handling this power without burning out. The non-linear properties of a mercury arc[5] or a cold cathode arc[6] have been exploited for the purpose of harmonic generation of submillimetre waves from a *Q*-band klystron, and it is suggested that the power handling capacity of the arc may be well suited to mixing optical maser signals. The output from each maser could be directed on to the cathode spot quite easily as the beam diameter is typically 2 mm. It seems conceivable that several watts of pulsed power could be generated in the far infra-red even if the mixing efficiency is only a few per cent.

In contrast to the ruby optical maser, the gas discharge type[7] is not continuously tunable over a wide band; nevertheless the exceptional spectral purity of the optical oscillation (which is many orders of magnitude narrower than in the ruby device) presents exciting possibilities as a source of beat frequency in the far infra-red. The gas optical maser has been operated at five different wave-lengths in the near infra-red and the strongest signal found at 11,530 Å. with a continuous wave output of 15 mW. The spectral purity has been measured to be in the region of 10–80 kc./sec. Thus beats only a few tens of kc./sec. wide between two optical masers operating at different spot frequencies should occur at wave-lengths of 180μ and 190μ as well as at several other wave-lengths between 15μ and 37μ. The beat frequency could probably be tuned over a narrow region by a small adjustment to the separation of the interferometer plates or by a variation of the gas pressure. Mixing of the optical signals could again be carried out by using a mercury or cold cathode arc as suggested before. Some sort of focusing would be necessary in this case. In this sort of way it should be possible to produce several tens of microwatts of continuous wave-power. Even this power-level, combined with the spectral purity of perhaps only a few kilocycles, presents a very attractive approach to the long-standing problem of suitable sources for high-resolution spectroscopy in the far infra-red.

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CHEMISTRY

A New Relation between the N—H Stretching Frequencies of Primary Amides

Bellamy *et al.*[1], from the data obtained for a number of aromatic primary amines in dilute solutions, have shown that the N—H asymmetric and symmetric stretching frequencies are related by the expression $\nu_s = 345{\cdot}5 + 0{\cdot}875\ \nu_{as}$. The standard deviation of the calculated symmetric stretching frequencies from those of the recorded values is about 5 cm.$^{-1}$. These workers also mention that the same relation would not be obeyed in situations where the two N—H links are non-equivalent.

We[2,3] have recorded the N—H stretching frequencies of a number of primary amides in dilute solutions in the region of 3,530, 3,420, 3,350 and 3,200 cm.$^{-1}$ and assigned the first two absorption bands to the N—H asymmetric and symmetric stretching frequencies of the monomers and the other two to the same modes of vibration of associated molecules. Davies and Hallam[4], Davies and Evans[5], and Cleverley[6] have recorded the N—H stretching frequencies of a series of primary amides in dilute solutions. The data thus available for fourteen primary amides yield a simple linear relation between the asymmetric and symmetric stretching frequencies of the monomers recorded in the regions of 3,530 and 3,420 cm.$^{-1}$. A least-squares treatment shows that the frequencies of these two free N—H stretching vibrations are related by the expression $\nu_s = 1{\cdot}1214\ \nu_{as} - 542{\cdot}5$. In cases where the frequencies are reported by more than one group of workers, the mean values of the frequencies are used for the least-squares treatment. This relationship is obeyed for bands in the regions of 3,530 cm. and 3,420 cm.$^{-1}$, the average deviation of the calculated values from those of the recorded being about 3 cm.$^{-1}$. This relation is indicated in Fig. 1. But the same empirical relation does not hold for bonded N—H

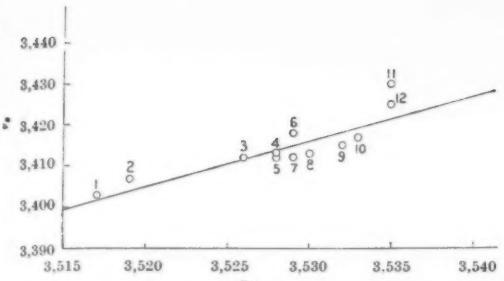

Fig. 1. 1, Trichloracetamide; 2, phenylacetamide; 3, *n*-valeramide; 4, *n*-butyramide; 5, *n*-decanamide and iso-butyramide; 6, acetamide; 7, *n*-dodecanamide and iso-valeramide; 8, *n*-octanamide; 9, *n*-heptanamide; 10, *n*-hexanamide; 11 benzamide; 12, formamide

asymmetric and symmetric stretching frequencies of the associated molecules in the region of 3,350 and 3,200 cm.$^{-1}$; the average deviation between the calculated and recorded ν_s frequencies is about 26 cm.$^{-1}$. This result is in agreement with our conclusions[3], since for the intermolecular associations proposed in our work on primary amides, it is considered that only one of the N—H links is bonded. The asymmetry thus introduced leads to the breakdown of this relationship.

The N—H stretching frequencies of four primary amides as recorded in solutions of dioxane[3] and the calculated ν_s values by the relation $\nu_s = 1{\cdot}1214\ \nu_{as} - 542{\cdot}5$ are given in Table 1.

Table 1. Calculated and Observed N—H Stretching Frequencies of Primary Amides in Dioxane (cm.$^{-1}$)

Amide	N—H stretching bands		
	Observed		Calculated
	ν_{as}	ν_s	ν_s
Formamide	3,443	3,357	3,318
Acetamide	3,450	3,360	3,326
Benzamide	3,450	3,360	3,326
n-Butyramide	3,450	3,360	3,326

The smaller values for the N—H stretching frequencies of the primary amides in solutions of dioxane have been explained by us[3] on the basis that the intermolecular associations break up considerably in this solvent and the hydrogen atom of the amino group of the monomer amides form association with the donor group of dioxane. From the observations in Table 1, it is seen that the relationship proposed by us breaks down for the N—H stretching frequencies of the primary amides in dioxane. The difference between the calculated and observed ν_s frequencies is about 35 cm.$^{-1}$. This result indicates that it may not be sterically possible for two molecules of dioxane to bond to the same amino group of the monomer amides. Similar results were obtained by Bellamy and others[1] in their work on amines in solutions of dioxane.

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Application of the Nickel Reaction and Thermal Isomerization of Alpha-Keto-Acid Hydrazones for their Chromatographic Analysis

The nickel reaction, discovered by Katsuki *et al.*[1], produces a red colour with pure *cis* isomers of α-keto-acid-2,4-dinitrophenylhydrazones and differentiates them from their corresponding *trans* isomers. We have found that this reaction applies to isomeric differentiation of the keto-acid hydrazones on paper chromatograms by the following procedures:

After chromatography of the pyruvic acid hydrazones in the dark on Whatman No. 1 filter paper, using tertiary amyl alcohol/ethanol/water 5: 1: 4 as the solvent, the chromatogram was sprayed first with a 5 per cent (w/v) solution of nickel chloride in 70 per cent aqueous ethanol followed by a fresh 0·3 per cent suspension of zinc powder in aqueous ethanol. The paper was heated at 100° C. for a few minutes and the higher spot of the *cis* isomer turned reddish, whereas the lower *trans* spot did not. The *cis* spots of glyoxylic, phenylglyoxylic, phenylpyruvic and *p*-hydroxyphenylpyruvic acid-2,4-dinitrophenylhydrazones gave similar responses. Concentration of nickel chloride and zinc powder in alcohol and the time of heating may have to be changed depending on the intensity of chromatographic spots and the stability of the hydrazone isomers. As a matter of fact, even *trans* spots give colour responses for this reaction, if they are readily isomerized by heat. If the *cis* hydrazone of pyruvic acid is dissolved in ethyl acetate and dried on the paper, it will give an orange colour, after spraying with alcoholic nickel chloride without application of zinc powder. This does not occur if alcohol is used as the solvent, and the *trans* isomer gives no colour with either solvent.

A second useful method in distinguishing isomers involves thermal isomerization to the stable isomer. For example, a crude hydrazone from the products of anaerobic glycolysis of the sarcoma 37 solid tumour homogenates upon chromatography gave two spots for the *cis* and the *trans* isomers of pyruvic acid hydrazone respectively. However, if the crude hydrazone were heated at 120° C. for 15 min. before chromatography, only the *trans* isomer was observed, owing to thermal isomerization. Thus, it was clear that both original spots were due to pyruvic acid alone and not to two keto-acids. This identification could be confirmed by the use of the nickel reagents which coloured one of the spots in the original chromatogram and left the single spot of the second chromatogram uncoloured.

Knowledge of thermal decarboxylation of dinitrophenylhydrazones of certain β-keto-acids as well as of thermal isomerization may be combined with the nickel reaction advantageously for greater certainty. For example, from mouse urine isomeric spots for pyruvic and phenylpyruvic acid hydrazones were readily identified by such a combination. Possible misassignment of spots of pyruvic acid hydrazone as being due to acetoacetic acid hydrazone, which has an R_F close to that of pyruvic acid hydrazone isomers, was avoided, since heating the crude hydrazone before chromatography produced only a spot for *trans* pyruvic acid and none for acetone hydrazone which would have been formed from acetoacetic by thermal decarboxylation[2]. Similarly, confusion between the hydrazone of oxaloacetic acid which decarboxylates to pyruvic acid on heating, and of

α-ketoglutaric acid, which does not decarboxylate, may be avoided. It should be noted that heating causes many aromatic keto-acid hydrazones to isomerize from *trans* to *cis*, the latter being the stable isomer in these cases, whereas the reverse behaviour is found with many aliphatic keto-acid hydrazones. The characterization of several aromatic keto-acid hydrazones will be presented elsewhere.

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A Molecular Beam Reactor for examining the Rate, and Primary Products, of Reactive Bimolecular Collisions

A molecular beam technique has been developed which permits investigation of the primary products of reactive collisions between gas molecules or radicals. The principle of the technique can be described by reference to the schematic diagram in Fig. 1. A molecular beam of one gas, A, is formed at an aperture, 1, in the wall of the vessel, 2, and collimated by a second aperture, 3. The emergent beam from 3 crosses the reaction zone, 4, and leaves by a third aperture, 5, to enter a detector, 6. The latter may conveniently be a mass-spectrometer, so orientated that the molecular beam of A crosses the ionization chamber of the mass-spectrometer without impinging on the electrodes. With sufficient pumping speed for the collimating, reaction and mass-spectrometer compartments, the mass-spectrum peaks due to A are produced almost entirely from molecules which have experienced a collision-free transit from aperture 1 to the ionization region, and the contribution from 'background gas' molecules of A in these compartments can be made negligibly small. The second gas, B, is admitted into the reaction compartment at a pressure such that the mean free-path of B in B in the main body of this compartment is of the order of the molecular beam path-length in this compartment. Now let n_B be the average number of molecules of B crossing unit area within this compartment in unit time, and let n_A be the average number of molecules of A which cross unit area normal to the molecular beam path, in unit time, in the reaction zone. Two limiting cases are conceivable. If $n_A \ll n_B$, then molecules of B will effuse according to the cosine distribution law into the mass-spectrometer compartment. Thus the B molecules detected there will have arrived there as a result of collisions with other B molecules in the reaction zone. On the other hand, if $n_A \gg n_B$ then any molecule of B in the reaction zone has a much greater probability of collision with a molecule of A than with another molecule of B. Thus each B molecule detected in the mass-spectrometer will have arrived there by a collision-free path

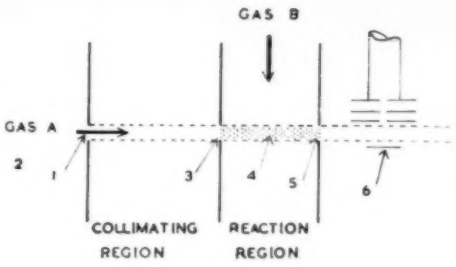

Fig. 1

from a collision with an A molecule in the reaction zone. As a first approximation the rate at which such molecules reach the detector is the same as for the case of molecular effusion in the absence of the beam of A molecules. If, therefore, A and B are molecular species which react together, it is possible to detect the primary products of the reactive collisions in the reaction zone.

This technique has been used for the study of the reaction of excited oxygen atoms with nitrogen dioxide :

$$O^* + NO_2 \rightarrow O_2 + NO \qquad (1)$$

The atomic oxygen was produced by an electrodeless radio-frequency discharge (10 Mc./sec.) through oxygen gas at 0·1 mm. mercury pressure in a tubular, hard-glass discharge tube. A round hole, 2·46 mm. diameter in the end of the tube, served as the first aperture, 1, of the molecular beam system. The molecular beam collimating system and the mass-spectrometer have been described elsewhere[1]. Mass-spectrometric study of the molecular beam showed that about 15 per cent of the oxygen molecules were dissociated into atoms, which were almost entirely in a metastable, electronically excited state with 2-eV. excitation energy. Nitrogen dioxide was introduced at a pressure about 10^{-4} mm. mercury into the reaction compartment (compartment C_2 of Fig. 3, ref. 1). Without the discharge operating, and using 70-eV. electrons for ionization in the mass-spectrometer, the ratio of the height of the $m/e = 46$ peak to that of the $m/e = 30$ peak from the nitrogen dioxide was 0·52. When the discharge was switched on, the ratio was reduced to 0·42, indicating formation of nitrous oxide by collisions of the atomic oxygen beam with nitrogen dioxide in the reaction zone. Exact quantitative assessment of the reaction efficiency of the collision process must await more precise measurements, but the preliminary figures suggest that the efficiency is considerably greater than the minimum limit of 1 reactive collision in 100 collisions proposed by Kistiakowsky and Kydd[2] for ground-state oxygen atoms.

While these preliminary results have served to demonstrate the potentialities of the technique, application is, at present, limited to collision processes having high reaction efficiencies. This restriction arises from the relatively small mass-spectrum peaks of reactant B, obtained with the technique described above. Preliminary experiments have shown that some improvement may be achieved by introducing reactant B also as a molecular beam aligned to intersect the beam of A in the reaction zone. Application of the technique to reactions with large activation energies may be possible by simulation of high-tem-

perature conditions by the use of fast molecular beams of reactant A.

We record our appreciation of the helpful discussions and continued interest of Dr. W. J. Dunning and Prof. F. H. Garner during the development of the technique. One of us (A. J. B.) is indebted to the Department of Scientific and Industrial Research for a research scholarship.

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Average Extinction Coefficient for Re-absorption of Fluorescence Radiation through Uranin Solution

The absorption and the fluorescence spectra of fluorescein in alkali solution overlap considerably. This overlap introduces a certain amount of error in the measurement of intensity of fluorescence in so far as it is not directly proportional to the incident radiation absorbed by the solution. If a fluorescent quantum is emitted somewhere deep in the solution, then during its passage from its point of emission to a measuring device like a photocell, there is a chance that it will be repeatedly re-absorbed and re-emitted by the solution. The consequence of this process is that the average life-time of the excited molecule is increased[1], as each absorption gives a fresh lease of life of about 10^{-8} sec. The effect will be greater, the higher the concentration of the fluoresecing solution. Since intensity of fluorescence is directly proportional to the life-time, τ, of the fluorescent molecule under a given set of conditions $\tau_1/\tau_2 = F_1/F_2$, the intensity as measured by the photocell will not be the true intensity. The effect has been explained in different ways[1-3].

To assess the effect of the absorption–re-emission process, it is desirable to know ε_2, the extinction coefficient for the re-absorption of secondary radiation by the experimental solution. The usual method for the determination of the extinction coefficient cannot be applied in the determination of ε_2 because of the polychromatic nature of the fluorescence radiation, the band-spread of which is nearly 1μ in the green region. A plot of log I_0/I versus C for a constant solution thickness, l, does not give a straight line as obtained for a solution obeying Lambert–Beer's law using monochromatic radiation. Besides this, the secondary re-emission process may also have some effect. That the latter effect is not so important can be shown by comparing measurements for a quenched and an unquenched solution under identical experimental conditions.

The Phoenix light-scattering instrument model 1,000, with single photomultiplier, was adapted for fluorescence measurements by inserting a filter transmitting 3650 Å. in the incident light beam. The photomultiplier was set at 90° to the incident beam. An alkaline fluorescein solution of appropriate strength ($1{\cdot}0 \times 10^{-4}$ M) was placed in a cell, 2 cm. wide and 3·7 cm. long. It was placed so that the incident beam fell just near the edge of the shorter side of the cell, exciting fluorescence radiation along the length of the solution. The longer side of the cell, facing the photomultiplier, was covered with black paper except for a small rectangular opening (0·3 mm. wide) to allow the fluorescent radiation to escape. This acted as a source of fluorescence radiation.

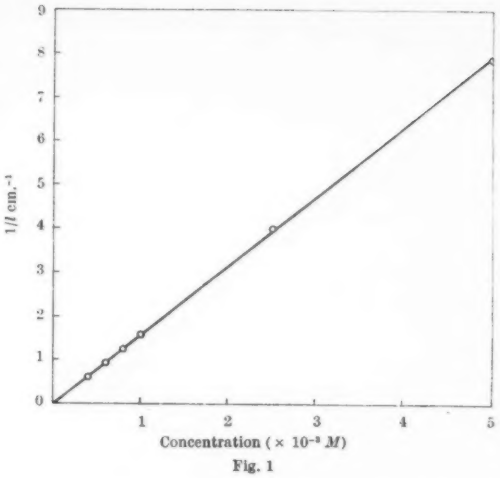

Fig. 1

Another cell of the same dimensions was placed in juxtaposition to the emitting cell, snugly between the latter and the photomultiplier. A yellow gelatine filter was placed in front of the photomultiplier to cut off any scattered primary radiation. The second cell contained alkaline fluorescein solution of various concentrations and the intensity of transmitted light was measured against water as the solvent blank. The plot of log I_0/I versus C was a curved line. The curvature was still there, when the same amount of potassium iodide solution was added to all the solutions to quench the fluorescence.

Now Lambert's law is known to have no exception. But for a diffuse, polychromatic and faint source like the fluorescence radiation, the use of cells of different dimensions to change the depth of the solution will mean introduction of an air gap between the source and the solution or between the solution and the photomultiplier. This will mean a loss of fluorescence radiation due to change in refractive index at the cell-air interface. To avoid this, the light-path was varied by inserting microscope glass-slides of known thicknesses into the 2-cm. cell placed next to the emitting cell. For each variation of light-path, l, readings were taken with the solvent blank (water) giving 100 per cent deflexion in the galvanometer. It was then replaced by the solution of known concentration, C, and the transmission of fluorescent light was measured in terms of galvanometer deflexion. For a given thickness, transmission was measured for different concentrations of the solutions ranging from 5×10^{-5} M to 1×10^{-2} M. By this technique, the thickness of the solution could be varied from 2 cm. to 0·05 cm.

For each concentration, the percentage transmission was plotted against the solution thickness. Curved lines were obtained, the curvature increasing with the concentration. The plot of log I_0/I against l was also curved because of the polychromatic nature of the radiation.

From a re-arrangement of Lambert–Beer's law we have :

$$\frac{1}{l} = \frac{\varepsilon_2 C}{\log I_0/I}$$

For a fixed percentage transmission, say 50 per cent:

$$\frac{1}{l} = \frac{\varepsilon_2 C}{\log 2} = \frac{\varepsilon_2}{0{\cdot}3010}\, C$$

Therefore, a plot of $1/l$ versus C should be a straight line passing through the origin. The slope of this line will be equal to $\varepsilon_2/0{\cdot}3010$ from which ε_2 can be calculated. The value so obtained will be the average value of the molar extinction coefficient over the whole wave-length range of fluorescence radiation.

The experimental data are plotted in Fig. 1. From the graph the slope of the straight line, $\varepsilon_2/0{\cdot}3010$ = 1,600 l.mole^{-1} cm.$^{-1}$ Therefore, ε_2 = 481·6 l.mole^{-1} cm.$^{-1}$.

This method takes advantage of the fact that the total number of absorbing centres throughout the experiment remains constant, so that all the interfering effects cancel out. Virtually, the experiment is carried out at a constant value of Cl in accord with the general principle of visual colorimeters. Further calculations show that the value of ε_2 so obtained is a function of the optical density of the measured solution.

We thank the Council of Scientific and Industrial Research, India, for the award of Senior Research Fellowship to one of us (G. S. S.) and also for sponsoring the above scheme. We also express our gratitude to Prof. S. N. Mukerjee, head of the Department of Chemistry, Jadavpur University, for his encouragement.

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Polarization of Triplet→Singlet Emission Spectra

Phosphorescence in homogeneous rigid media was identified by Lewis, Kasha and Calvin[1] during 1941–45 as triplet→singlet emission spectra. Simultaneously Weisman and Lipkin[2] were able to show by a wide-angle interference technique that these emissions are electric dipole transitions. Following these investigations, selection rules for triplet–singlet transitions, based on the triplet states undergoing spin-orbit coupling due to the spin magnetic moment of the electrons interacting with the molecular field, were given by McClure[3] and placed on a rigorous basis by Weisman[4]. Recently, Hutchison and Mangum[5], by observing paramagnetic resonance absorption in the phosphorescent state of naphthalene, have confirmed the triplet assignment.

The triplet–singlet transitions, forbidden by multiplicity selection rules, can be made allowed by the spin-orbit perturbations, which mixes states of different multiplicity. Thus the emitting triplet state, T, can be perturbed by one or more of the singlet states, S_k, through spin-orbit coupling and acquire a singlet character, the transition to the ground-state, S_0, being allowed by this singlet character. Since the components of the spin-orbit coupling operator, (grad $\mathbf{V} \times \mathbf{P}$) · $\mathbf{S}$, transform like the components of the rotation operator, S_k should have the same symmetry as the product of symmetries of T and the rotation operator. According to this scheme the $T \rightarrow S_0$ spectrum should be polarized parallel to the $S_0 \rightarrow S_k$ absorption. Further, it should be parallel or perpendicular to other singlet–singlet transitions in the molecule depending on whether they are parallel or perpendicular to $S_0 \rightarrow S_k$. This can be confirmed directly, in favourable cases, by measuring the relative polarization of triplet→singlet bands with respect to any two mutually perpendicularly polarized singlet–singlet absorption bands in the molecule. While there is strong indirect evidence[1] from life-time measurements and heavy-atom effects for spin-orbit coupling perturbations in molecules, there is no direct confirmation of the mixing of the triplet with the singlet states, since polarization work up to now[6,11] has involved molecules where unambiguous theoretical prediction and experiment have not been possible.

Pyrazine, , is well suited for such an experiment for the following reasons. (1) the triplet→singlet emission has been assigned beyond doubt[7] as $^3B_{2u}(n,\pi^*) \rightarrow {}^1A_g$. (2) The two singlet–singlet transitions at 3300 Å. and 2600 Å. corresponding to $^1B_{2u}(n,\pi^*) \leftarrow {}^1A_g$ and $^1B_{2u}(\pi,\pi^*) \leftarrow {}^1A_g$ are well separated and their polarizations are known to be respectively along the axis perpendicular to the plane of the ring[8]; and perpendicular to the N—N axis but in the plane of molecule[9]. (3) It is predicted[10] unambiguously from the rotational nature of the spin-orbit coupling operator that all planar azine $\pi^* \rightarrow n$ triplet→singlet emissions should be polarized in the plane of the molecule, and in the case of pyrazine the nodal properties of the π^* and n orbitals lead to the prediction that the triplet→singlet emission should be polarized along the N—N axis[10], that is, perpendicular to both the singlet–singlet transitions at 3300 Å. and 2600 Å.

We have determined the polarization of the triplet→singlet emission spectrum throughout the band system relative to the $^1B_{2u}(n,\pi^*) \leftarrow {}^1A_g$ and to the $^1B_{2u}(\pi,\pi^*) \leftarrow {}^1A_g$ absorptions. The method consists in exciting into each of the absorption band systems using narrow band monochromatic polarized light at 77° K. in thin (1–3 mm. thick) rigid media and measuring the polarization of the resulting phosphorescence. Great care has been taken to reduce rotational depolarization, and under the experimental conditions this is small in spite of the long life-time of the phosphorescence. The degree of polarization, defined as $P = \dfrac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$, where $I_{\parallel}$ and $I_{\perp}$ are intensities of the emitted radiation parallel and perpendicular to the electric vector of the polarized exciting light, theoretically is 0·50 if the emission is polarized parallel to, and − 0·33 if it is polarized perpendicular to, the polarization of the exciting radiation[11]. In pyrazine for $^1B_{2u}(n,\pi^*) \leftarrow {}^1A_g$ and $^1B_{2u}(\pi,\pi^*) \leftarrow {}^1A_g$ excitations, P for the $^3B_{2u}(n,\pi^*) \rightarrow {}^1A_g$ phosphorescence emission is found to be − 0·26. The deviation from the ideal value of − 0·33 is probably due to depolarization factors in the optical system and in the rigid media and not due to the inherent nature of the emission. The above results leave only one possibility for the polarization of the triplet→singlet emission of pyrazine: along the N—N axis of the molecule, confirming the predicted spin-orbit coupling scheme in this molecule.

Further, experiments on the pyrimidine $\pi^* \rightarrow n$ phosphorescence emission also conclusively yield in plane polarization, conforming to the spin-orbit coupling prediction. A detailed account of this work will be published in the *Journal of Chemical Physics*.

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Deterioration of Solid-coated Capillary Columns on Standing

THE recent introduction of capillary columns, which are able to give efficiencies of up to 1,000,000 theoretical plates[1], has given a great stimulus to the technique of gas chromatography. Many separations, heretofore impossible with packed columns, are extremely simple when capillary columns are used.

Although we have generally been successful with capillaries, we have recently noted a disturbing phenomenon when using them. The phenomenon referred to is the fact that at least some solid-capillaries will undergo a severe diminution of column efficiency on standing.

Fig. 1 shows the performance of a newly prepared 60 phenoxythiin capillary column. The column was prepared by coating a 0·010 in. internal diameter stainless steel capillary with 10–12 mgm. of phenoxythiin. 10 ml. of a 10 weight per cent solution of phenoxythiin in ethylene chloride were passed slowly through the column under a pressure of 10 p.s.i.g. of nitrogen. After the excess solution had all passed through the capillary, the flow of nitrogen was maintained overnight, at room temperature. The column was then conditioned for 4 hr. at 60° C. and then put into service, with the result shown in Fig. 1. The column continued to give equivalent performance for four weeks, during which time at least fifty samples were analysed on it.

The column was then taken from the chromatograph and stored (at room temperature) for six months. Both ends of the column were opened to the atmosphere during storage.

On attempting to re-use this column, we noted the extreme deterioration of efficiency shown in Fig. 2, which is a run made under identical conditions, with the same column and the sample as the run shown

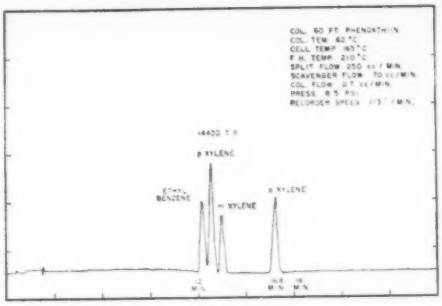

Fig. 1

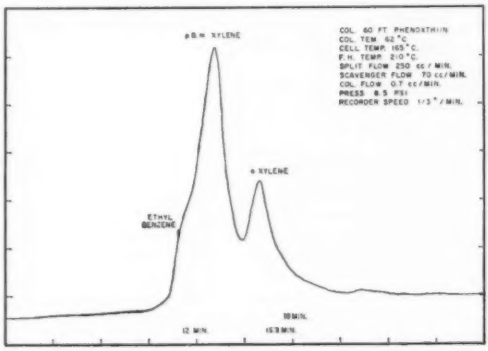

Fig. 2

in Fig. 1. While the lack of resolution in Fig. 2 makes it difficult to calculate column efficiency exactly, a rough calculation indicates that the efficiency has dropped from 14,400 theoretical plates in Fig. 1 to not more than 200 theoretical in Fig. 2.

A similar behaviour has been observed with columns coated with 7,8-benzoquinoline, another solid material. The deterioration of column efficiency was as extreme with this material as with the phenoxythiin.

We are at a loss to give a full explanation for this phenomenon. Two possibilities present themselves: (*a*) both the phenoxythiin and the 7,8-benzoquinoline were exposed to atmospheric oxygen during the standing period for at least six months and were hence subject to oxidation or other processes of decomposition; (*b*) on removal of the column from the heated chromatographic oven, and subsequent cooling, the substrates may have recrystallized in such a fashion that on re-melting they formed globules instead of a uniform coating. In support of the second possibility, we have noted that the appearance of Fig. 2 is strikingly similar to chromatograms made with glass capillaries, where we could observe the presence of substrate globules.

Fortunately, this behaviour appears to be restricted to solid-coated capillaries. We have not observed it in any of the liquid-coated (squalane, tri-cresyl phosphate, dioctyl sebacate) capillaries we have made. While it would be premature to predict such behaviour for all solid substrates, it does appear that this is a point worth further investigation, since phenoxythiin and 7,8-benzoquinoline are two

of the most effective separators yet found for the lower aromatics.

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Adducts of Urea and Thiourea obtained by means of a Sublimation Technique

The classical methods for obtaining adducts of urea and thiourea with some classes of organic substances imply the use of aqueous or alcoholic solutions of 'reagents' (urea or thiourea). Other methods have been described more recently by which adduction is obtained by mixing solid urea directly with the proper 'reactant'[1].

It has been found that the adduction of the urea-hydrocarbon type is actually a two-phase process involving urea in the solid state. The solvents for urea seem to have no function other than that of precipitating the reagent in the finely divided form suitable for interaction with the hydrocarbon[2].

All the adduction processes with solid urea usually require an appreciable induction time, which is necessary for the transition of the reagent from its normal crystalline form to that of the adduct including the reactant molecules. This induction time is usually shortened by reducing the urea in minute particles and adding small quantities of acetone or other simple ketones. The catalytic action of acetone is explained by way of having a part in the formation of an intermediate unstable adduct with urea[3].

In order to perform an adduction process with highly divided solid reagent, avoiding any mechanical stress on the adduct, we directly condense sublimated urea (or thiourea) on the liquid reactant placed on a cooled surface. The sublimated urea and thiourea, when in contact with an adductible material, crystallize around the matrix molecules as adduct, while, if some non-adductible material is placed on the cold surface, recondensation of urea and thiourea takes place in the normal crystalline form (tetragonal and orthorhombic respectively).

The process, which is actually rather fast, may be easily followed and successfully used for analytical purposes making use of the differences between the infra-red spectra of urea (and thiourea) in the normal crystalline form and in the adduct form[4–7]. The infra-red spectrum of the suspension obtained through adduction (in excess of reactant) clearly reveals whether an adduction has taken place. The spectrum may show also an excess of reactant, which in general does not disturb the detection of the main bands of the adduct. The excess reactant, anyway, can be washed away with pentane. In most cases the potassium bromide (sodium chloride for thiourea) pressed disks technique can be used without decomposition of the adducts.

The present technique of adduction 'via sublimation' may be performed with high-boiling adductible liquid molecules in a conventional laboratory sublimator using the vacuum supplied by a rotary oil pump. At a pressure of 0·1 mm. mercury, the experimental temperature is about 120° C. for urea and 140° C. for thiourea.

I have used this technique with a number of compounds. With urea I have adducted straight-chain paraffins, saturated and unsaturated fatty acids and esters as well as some alcohols and ketones. With thiourea I adducted several branched paraffins and naphthenic hydrocarbons.

The method is useful for the investigation of the adductibility of pure substances, thus providing information about their molecular shapes. The quantity of the product required is about 1 mgm. or less.

Furthermore, it is possible to detect small quantities of adductible materials in complex mixtures. For example, in a 1 per cent mixture of cetane in decaline the sublimated urea condensates in the hexagonal form (adduct), apparently until molecules of cetane are available. Then the condensation of urea takes place in the normal tetragonal form. This fact allows us to extend the investigation to solid materials (for example, higher hydrocarbons and their hydroxylated or carboxylated derivatives), when dissolved in a non-adductible high-boiling liquid.

Care must be taken to avoid in this case the deposition of a too thick layer of sublimated urea (or thiourea) on the liquid surface. As non-adductible solvents I used a high-boiling naphthenic oil for urea and an aromatic oil for thiourea. Although in this case we can find, besides urea or thiourea as adducts, even some reagent in the normal crystalline form, the complexing of the adductible fraction dissolved in the oil may be detected from the inspection of the shape of some characteristic band in the infra-red spectrum of the resulting suspension.

The regions which appear more characteristic for this inspection are near 3,400 cm.$^{-1}$ (ν(N—H) vibrations), near 1,650 cm.$^{-1}$ ($\delta(NH_2) - \nu(C{=}O)$ vibrations for urea and $\delta(NH_2)$ vibration for thiourea) and in the 700–800 cm.$^{-1}$ range (ν(C—C—N) for urea and $\nu(C{=}S) - \nu(N—C—N)$ for thiourea), as can be seen

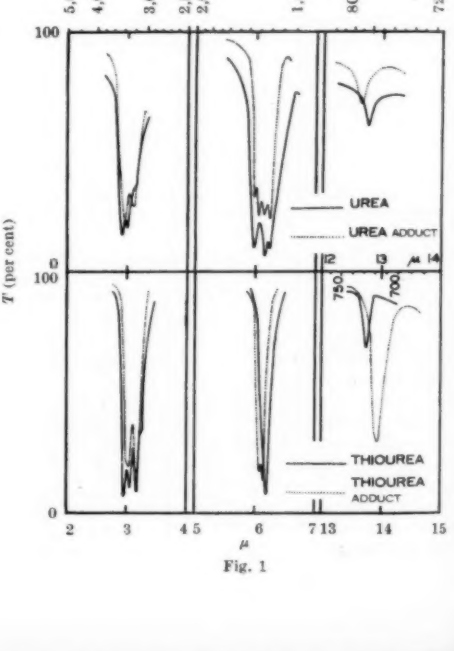

Fig. 1

from Fig. 1. The 3,400 cm.$^{-1}$ region is particularly suitable when hydrocarbons or any of their derivatives not containing —OH or —NH— groups are investigated about adductibility versus urea or thiourea or are employed as solvents for solid materials.

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BIOCHEMISTRY

Chromatographic Separation of Biologically Active Components from Commercial Trypan Blue

Trypan blue has been shown to produce reticulosis and reticulo-sarcoma as well as congenital malformations when injected subcutaneously into rats[1,2]. Considerable differences in the biological activity of various commercial preparations of trypan blue have been found, and it has been suggested that these are due to variations in the amount and kind of impurities present[3,4]. Commercial trypan blue is known to contain a red impurity which is more diffusible than trypan blue itself and which is excreted rapidly in the urine[5–7]. This material has been separated from trypan blue by extraction with ethyl alcohol[6] or with acetone[7] or by passing through a cellulose column[7,8]. Although this red fraction causes some resorptions, when injected into pregnant rats, it does not appear to produce congenital malformations[4,8]. This suggests the possible presence of other active impurities in trypan blue.

We have fractionated commercial trypan blue (Merck) on alumina and have separated a purple impurity in addition to the red fraction. The former was highly active in inducing reticulosis in rats, whereas the latter was inactive. Purified trypan blue itself showed only slight activity. The crude dye (6 mgm. in 6 ml. methylethylketone/water/ethanol 75:25:10) was placed on a column (12 × 2·2 cm.) of alumina (B.D.H. or Merck, 50 gm.) and eluted successively with methylethylketone/water/ethanol (75:25:10), water and 0·01 *N* sodium hydroxide. The methylethylketone/water/ethanol eluted first a reddish fraction ($\lambda_{max.}$ 560mμ), followed by the main blue fraction ($\lambda_{max.}$ 600 mμ). Water eluted a single purple fraction ($\lambda_{max.}$ 570 mμ) as did 0·01 *N* sodium hydroxide. The contribution of the various fractions to the absorption of the crude dye at 580 mμ is shown in Table 1.

The red and blue fractions each gave a single band when rechromatographed on alumina. The former could, however, be resolved into two components, a faster moving red-violet dye and a slower blue-violet dye, by paper chromatography with 80 per cent aqueous ethanol as solvent. In this system the blue fraction moved very slowly and the purple fraction not at all. On rechromatographing the purple fraction on alumina, approximately half the material could be eluted with water; the remainder only came off with 0·01 *N* sodium hydroxide. It thus appears as if the sodium hydroxide fraction were derived from the water fraction as a result of irreversible adsorption of the dye on alumina.

Table 1. Relative Contribution of Various Fractions to the Optical Absorption of Crude Trypan Blue at 580 mμ

	Per cent
Red (violet)	12
Blue	76
Purple (water fraction)	5
Purple (sodium hydroxide fraction)	7

In order to prepare sufficient of the various fractions for biological testing 10 gm. of Merck's trypan blue was applied as a 0·5 per cent solution in methylethylketone/water/ethanol (75:25:10) to an alumina column (12 × 4·5 cm.) Under these conditions the violet components (red fraction) passed through the column without hold-up, while the blue fraction was retained until the column was saturated with blue dye. It was then eluted with methylethylketone/water/ethanol solvent. The purple fraction was finally eluted with water, and the material which remained on the column was discarded.

After removal of the solvent, each of these fractions was tested for its power to induce proliferation of the reticulo-endothelial system of rat liver, in a group of five female rats of the same age. The red fraction was used as a 0·5 per cent solution, and the blue and purple fractions as 1 per cent solutions in distilled water. Each rat received two subcutaneous injections of 1 ml. at an interval of a fortnight, and all rats were killed seven days after the last injection, that is 21 days after the beginning of the experiment. A further group of five rats served as controls and was treated with crude trypan blue (1 per cent) in the same manner.

No observable reticulosis developed in any of the five rats treated with the red fraction. The blue fraction caused a mild portal reticulosis which was very much less marked than that observed in rats treated with crude trypan blue. In each of the five rats the purple fraction produced an intense portal as well as an intralobular reticulosis associated with the formation of numerous small cysts in the portal tract. In some regions, there was increased vascularity between the small cysts, leading to collapse of the cysts and accentuation of the portal reticulosis. The reticulosis produced by the purple fraction was of far greater intensity than that observed in the animals treated with the unfractionated trypan blue.

From these results it appears that the activity, so far as proliferation of the reticulo-endothelial cells of rat liver is concerned, is associated with the purple fraction. The chromatographic behaviour of this active fraction differs considerably from that of the well-known red fraction, which was separated by 80 per cent ethanol into two components and which appears to be inactive with respect to induction of reticulosis as well as to production of congenital malformations. Trypan blue itself has only slight activity ; but the possibility that its activity is due to contamination with purple dye cannot be excluded.

Elucidation of the structures of the various components of trypan blue is in progress.

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Stimulation of Lipogenesis in Thyroid Tissue *in vitro* by Free Fatty Acids

Recently, it has been demonstrated that some of the *in vitro* actions of certain pituitary hormones on adipose tissue can be reproduced by simple supplementation of the suspending medium with albumin-complexed free fatty acids[1]. This has prompted a more generalized analysis of the effects of free fatty acids on the metabolism of surviving preparations of target endocrine glands. We have incubated slices of sheep and human thyroid tissue at 38° C. and *p*H 7·4 in modified Krebs–Ringer *tris*-phosphate or Krebs–Ringer bicarbonate[2] containing 2 mgm./ml. of uniformly labelled glucose (glucose-U-^{14}C), 10 mgm./ml. of fat-free bovine albumin[3], and varying quantities of sodium oleate. Direct chemical analysis[4] disclosed that such exogenous supplementation with oleate augmented the thyroidal pool of free fatty acids. The associated striking increases in lipogenesis from glucose-U-^{14}C form the basis of the present report.

Following 3 hr. incubation, thyroid slices were frozen with liquid nitrogen and extracted for total lipids as described previously[9]. Washed extracts were partitioned into neutral lipids and phospholipids with silicic acid columns[5] and aliquots of the fractions were assayed for radioactivity with an automatic Packard 'Tri-Carb' liquid scintillation counter (Packard Instrument Co., Inc., La Grange, Ill.). The remaining portions were hydrolysed for 6 hr. at 100° C. with 6 *N* hydrochloric acid–50 per cent ethyl alcohol. Fatty acids and non-saponifiable lipids were removed by three extractions with petroleum ether and pooled ether extracts were washed, evaporated to dryness and re-suspended for radioactive assay. The aqueous residues of the hydrolysates were evaporated *in vacuo* and re-suspended in water for resolution on paper by ionophoresis (*p*H 3·6; pyridine–acetic acid–water 1 : 10 : 89 v/v) and by chromatography (ethanol–water 8 : 1 v/v). Radioactive moieties on ionophoretograms and chromatograms were localized by radioautography and appropriate segments of paper were cut out for direct counting in the liquid scintillation counter. Recovery standards of glycerol-1-^{14}C were processed with each experiment to correct for losses of glycerol through volatilization and degradation[7] during preparative manipulations.

In Krebs–Ringer bicarbonate as well as Krebs-Ringer *tris*-phosphate, total lipogenesis from glucose-U-^{14}C by surviving thyroid slices was augmented two- to three-fold in the presence of 1 μM/ml. of albumin-complexed oleate. The stimulation was largely effected by a four- to five-fold increase in the radioactivity of the neutral lipid fraction (Table 1). Conjoint analyses to be presented elsewhere indicated that the enhanced lipogenesis was accompanied by a small rise in oxygen consumption, an inconstant alteration in glucose-assimilation, a reduction in the oxidative decarboxylation of glucose-U-^{14}C, and an increased formation of lactic acid-^{14}C from the labelled glucose. Less-prominent, albeit still significant, increases in the incorporation of glucose into thyroid lipids were obtained with higher concentrations of oleate (4–6 μM/ml.) which depressed Qo_2. Analysis of aqueous residues following acid hydrolysis disclosed that glycerol-^{14}C (as glycerol or glycerophosphate) accounted for more than half the lipid radioactivity. Formation of glycerol from glucose-U-^{14}C was enhanced by 1 μM/ml. oleate; the increase was chiefly channelled into the neutral lipid fraction (Table 2). Oleate also stimulated the incorporation of radioactivity into neutral lipid components which retained solubility in petroleum ether following acid hydrolysis (Table 2). Alkaline hydrolysis disclosed that this increase consisted principally in fatty acids rather than non-saponifiable lipids.

From all the foregoing, it is apparent that exogenous supplementation of intra-thyroidal fatty acids results in striking changes in lipogenesis. The

Table 1. Effects of Albumin-complexed Oleate on the Incorporation of Glucose-U-^{14}C into the Lipid Fractions of Thyroid Slices

Exp. No.	Species	Medium†	μgm. glucose-U-^{14}C/gm. initial weight to:*			
			Phospholipid		Neutral lipid	
			A	*B*	*A*	*B*
1	Lamb	KRT-P	26·5	30·8	6·8	48·7
2	Lamb	KRB	31·6	38·7	9·2	42·1
3	Lamb	KRT-P	26·7	26·0	10·5	55·0
4	Lamb	KRT-P	41·4	42·6	14·8	86·0
5	Human	KRT-P	28·8	34·5	19·7	93·9
6	Human	KRB	24·9	19·1	17·4	48·2

* Results are expressed on the basis of the initial specific activity of the glucose-U-^{14}C in suspending media and the recovery of radioactivity as phospholipid of neutral lipid in thyroid slices following incubation.
† Thyroid slices were incubated for 3 hr. in modified Krebs–Ringer *tris*-phosphate (KRT-P) or Krebs–Ringer bicarbonate (KRB) containing fat-free albumin (10 mgm./ml.), uniformly labelled glucose (2 mgm./ml.), and no free fatty acids (*A*) or 1 μM/ml. of sodium oleate (*B*).

Table 2. Distribution of Radioactivity in the Hydrolysis Products of Thyroid Lipid Fractions

Exp. No.*	μgm. glucose-U-^{14}C/gm. initial weight to: Phospholipid				Neutral lipid			
	Petroleum ether		Glycerol		Petroleum ether		Glycerol	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*
1	1·4	0·6	19·3	25·9	1·9	7·4	3·7	24·1
2	4·0	0·9	20·2	25·1	3·7	13·6	5·2	16·3
5	1·6	0·7	—	—	7·0	17·6	11·6	55·9
6	2·1	1·0	—	—	6·8	19·5	9·5	26·2

* Experiments are the same as in Table 1. Phospholipid and neutral lipid fractions were hydrolysed with 6 *N* hydrochloric acid–50 per cent ethyl alcohol. Acid hydrolysates were resolved into radioactive components which retained solubility in petroleum ether or which exhibited the chromatographic and ionophoretic properties of glycerol or glycerophosphate (glycerol). The (*B*) or (*A*) denotes the respective presence or absence of sodium oleate in suspending media during incubation.

phenomenon is somewhat similar to the "experimental aberrant lipogenesis" which has been described by Cogan and Kuwabara following 24–48 hr. of incubation of tissue 'buttons' with serum and sodium oleate[7]. However, in their system, increased sudanophilic material was not observed with thyroid preparations.

For the moment, the biochemical basis for the effects of albumin-complexed oleate must remain conjectural. The existence of intra-thyroidal mechanisms for the activation of fatty acids may be inferred from the finding in this Laboratory that surviving sheep thyroid slices can esterify and oxidize albumin-complexed palmitate-^{14}C (ref. 2). Unless such activation were rate-limiting, it is conceivable that an increased availability of free fatty acids could abet lipogenesis in several ways. First, it could provide long-chain coenzyme A derivatives for esterification of glycerophosphate[8]. Indeed, the plethora of acylated fatty acids might also compete successfully with cytidine derivatives for the unesterified carbon of the α,β-diglycerides[8] and thus account for the predominant impact on the neutral lipid fraction. Since phospholipid components were not fractionated in the present work, one cannot assess whether the occasional modest stimulation of phospholipid radioactivity proceeded beyond phosphatidic acid. Secondly, oxidation of an increased quantity of activated fatty acids could indirectly promote the synthesis of fatty acids as well as glycerophosphate from glycolysis. The generation of reduced diphosphopyridine nucleotide during catabolism of fatty acids by β-hydroxyacyl dehydrogenase[9] would provide co-factors for the former[10], and would enhance the formation of the latter from dihydroxy acetone phosphate since the equilibrium constant of soluble glycerophosphate dehydrogenase already favours this direction[11]. Differentiation between the above speculative possibilities (that is, metabolic changes secondary to the disposition of free fatty acids) and other potential actions of fatty acids[12] (that is, surface-active effects which may not entail prior activation) must await further investigation.

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Biosynthesis of Polyenoic Fatty Acids in Human Whole Blood

It has been known for some time now that human blood cells are able to incorporate acetate-1-^{14}C into blood lipids[1,2]. James *et al.*[2,3] found the incorporation of radioactive acetate into the saturated as well as into the unsaturated fatty acids of blood lipids, including the 'essential fatty acids'. Based on these experiments, the authors concluded that a total synthesis of essential fatty acids in human blood would be possible. Klenk[4] and Mead *et al.*[5] showed that the C_{20}- and C_{22}-polyenoic fatty acids in rat liver originate from exogenous linoleic and linolenic acids by extension of the carbon chain by acetate units starting at the carboxylic group. In addition, new double bonds are introduced in the divinylmethane-rhythm in direction towards the acid group. This mechanism is valid for polyenoic acid synthesis in the human liver[6] as well as in the descending classes of vertebrates[7].

In order to find out if polyenoic acids can be synthesized by blood cells in the same way we have incubated human whole blood with acetate-1-^{14}C. The fatty acids were isolated and the unsaturated fraction degraded by oxidative ozonolysis. The resulting dicarboxylic acids were separated by column chromatography and the activity measured in a windowless flow-counter. Results are shown in Table 1 (for methods see refs. 4, 6, 7).

Radioactivity was found almost exclusively in glutaric acid (predominantly from Δ5,8,11,14-eicosatetraenoic acid = arachidonic acid), succinic acid (predominantly from Δ4,7,10,13,16,19-docosahexaenoic acid) and in the higher dicarboxylic acids, mainly azelaic acid (from oleic, linoleic and linolenic acids). On the contrary, malonic acid originating from the central parts of the polyunsaturated carbon chains showed only minute activity. This low activity seems to exclude the total synthesis of linoleic and linolenic acids which might be concluded from the activity of azelaic acid. Although degradation and re-extension reactions at the carboxylic end of linoleic and linolenic acids could contribute to the incorporation of this activity, it will originate mainly from oleic acid.

The results strongly support the mode of formation of polyenoic fatty acids outlined here, which seems also to be valid for their biosynthesis in human blood. We hope to obtain further support of these results by reductive ozonolysis of the unsaturated fatty acids

Fig. 1. Relation between total activity of fatty acids from human whole blood after incubation with acetate-1-^{14}C and leucocyte count

Table 1. SPECIFIC ACTIVITY OF DICARBOXYLIC ACIDS FROM OXIDATIVE OZONOLYSIS OF UNSATURATED FATTY ACIDS FROM HUMAN WHOLE BLOOD AFTER INCUBATION WITH ACETATE-1-^{14}C

In each case 50 ml. of freshly drawn blood were heparinized and after addition of 15 mgm. glucose and 0·23 mc. = 8·35 mgm. acetate-1-^{14}C incubated for 5 hr. at 37°. Yields in mole per mole polyenoic acid; specific activity in counts/min. and mgm.

Experiment	Higher M.W. dicarboxylic acids (azelaic acid)		Glutaric acid		Succinic acid		Malonic acid	
	Yield	C.p.m./mgm.	Yield	C.p.m./mgm.	Yield	C.p.m./mgm.	Yield	C.p.m./mgm.
1	0·54	1,275	0·12	655	0·07	532	0·15	28
2	0·51	4,230	0·12	1,240	0·08	1,195	0·43	62
3	0·60	5,640	0·12	2,890	0·06	2,290	0·36	85
4	0·59	3,810	0·10	1,980	0·08	1,620	0·32	68

and subsequent activity measurement of the degradation products derived from the methyl end[7].

It was suggested previously that the fatty acid synthesis in human blood occurs predominantly in erythrocytes. More recently several authors have given evidence for lipid synthesis mainly in leucocytes[8–10]. Besides, these platelets[10] and reticulocytes[9,10] seemed to be involved in fatty acid synthesis too. In the course of our investigations we obtained evidence for synthesis of fatty acids in human blood linked predominantly to leucocytes. Since our experimental conditions for the incubation of blood from subjects with different leucocyte counts were kept constant, we were able to compare leucocyte count and incorporation of radioactivity into the total fatty acids (Fig. 1).

Surprisingly enough we found good linearity between leucocyte count and total incorporated activity.

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Oxidation-Reduction Reactions of Plastoquinone in Isolated Chloroplasts

QUINONES have previously been implicated as oxidation-reduction carriers in the photosynthetic electron transport system on the assumption that chloroplasts of higher plants contained vitamin K_1[1–3]. It has now been shown that chloroplasts contain little or no vitamin K_1 but that another quinone, 2,3-dimethyl-5-solanesyl-benzoquinone, is present in relatively large amounts[4–6]. This quinone is known as plastoquinone, Q_{255} and Kofler's quinone.

Evidence that plastoquinone functions in the electron transport system associated with photosynthesis has been presented by Bishop[6], who has shown that it will restore photolytic activity when added to spinach chloroplasts which have been extracted with organic solvents. Recently, Crane *et al.*[7] have claimed that exposure of spinach chloroplasts to light resulted in an 80 per cent reduction of the endogenous plastoquinone. In the present communication we provide some further observations on the oxidation-reduction reactions of endogenous plastoquinone in isolated chloroplasts.

Chloroplasts were isolated from sugar-beet leaves (var. Sharp's Klein Wanzleben *E*) by the method of Mayer and Friend[8], except that the isolation medium was buffered to *p*H 7·8 with 0·05 *M* sodium/potassium phosphate instead of *tris*-hydrogen chloride. The chloroplasts (0·4–0·8 mgm. chlorophyll/ml.) were suspended in a medium containing 0·4 *M* sucrose, 0·01 *M* potassium chloride and 0·05 *M* sodium/potassium phosphate, *p*H 7·0.

Plastoquinone was extracted by a modification of the method[9] used for the extraction of ubiquinone from mitochondrial preparations, and the oxidation-reduction state of the quinone measured by the change in extinction at 255 mμ after the addition of sodium borohydride.

The concentration of plastoquinone in the chloroplasts was 9 μmoles/gm. protein or 0·1 mole per mole chlorophyll. This concentration agrees with that determined in spinach chloroplasts by Crane *et al.*[7]. There was no evidence for the presence of vitamin K_1. It is interesting to compare this concentration with that of another electron carrier in the chloroplast, cytochrome *f*. It has been shown[10,11] that the molar ratio of chlorophyll to cytochrome *f* in a number of chloroplast preparations is about 200–400. Thus the molar concentration of plastoquinone exceeds that of cytochrome *f* by a factor of 20–40. In this connexion, it is interesting to note that a related quinone, ubiquinone (coenzyme Q), in mitochondria is also present in a considerable molar excess over the individual cytochromes in mitochondria.

The oxidation-level of plastoquinone (that is, percentage of total in oxidized form) in freshly isolated chloroplasts varied between 45 and 90 per cent; the oxidation-level appeared to increase as the chloroplasts aged. Illumination (5 min. at 3,200 ft.-candles) resulted in a fall of the level to 35–65 per cent. Thus there had been approximately only 15 per cent reduction of the quinone. Crane *et al.*[7], on the other hand, have reported an 80 per cent reduction in a spinach chloroplast preparation incubated under similar conditions. The reason for this discrepancy is not apparent at the moment.

In order to obtain further information on the oxidation-reduction reactions of plastoquinone and its site of action in the electron transport system, the effect of certain natural and artificial electron carriers was studied.

Addition of 2,6-dichlorophenolindophenol (final concentration 0·5 m*M*) to the chloroplasts in the dark converted all the plastoquinone into the oxidized form. When this mixture was illuminated there was

Table 1. Reduction of Endogenous Plastoquinone in Chloroplasts by TPNH

Addition	Incubation: Time	Incubation: Light or dark	Plastoquinone (per cent in oxidized form)
Experiment 1			
None	0	dark	45·8
TPNH	5 min.	dark	26·0
TPNH	5 min.	light	34·3
Experiment 2			
None	0	dark	87·5
None	5 min.	light	73·5
TPN + chloroplast extract	15 min.	dark	80·5
TPN + chloroplast extract	15 min.	light	59·0

The incubation mixture contained 1 ml. chloroplast suspension, 1 ml. chloroplast extract or water, 0·625 m*M* TPN or TPNH.

5 per cent reduction to the quinol. Thus, under conditions where the Hill reaction is operative, the steady-state level of the plastoquinone is 95 per cent oxidized.

It was found that incubation of the chloroplasts with reduced triphosphopyridine nucleotide (TPNH) in the dark resulted in a reduction (20–30 per cent) of the plastoquinone. A similar reduction could also be brought about by TPNH formed photochemically in a reaction mixture consisting of chloroplasts, triphosphopyridine nucleotide (TPN) and a chloroplast extract containing photosynthetic pyridine nucleotide reductase[12] (Table 1).

These results provide further evidence that plastoquinone is a functional member of the photosynthetic electron transport system. The site of action of the quinone is now under investigation.

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Metabolites of Vitamin B_{12} in Bile after Parenteral Administration of the Vitamin

Several investigations have shown that, in animals and in man[1], the major proportion of the total radioactivity excreted after an injection of radioactive vitamin B_{12} is excreted in the bile, and that in man at least two-thirds of the biliary radioactivity is re-absorbed from the intestine. This indicates that vitamin B_{12} is subject to an entero-hepatic circulation similar to other substances in bile.

Radioactive bile was collected via a bile duct cannula from rats injected with radioactive vitamin B_{12}. Vitamin B_{12} and radioactive cobalt-containing metabolites of B_{12} were extracted from the bile. The acidified bile (*p*H 2–3) was denatured with 95 per cent ethanol, and any bound B_{12} or B_{12} analogues freed by boiling in water for 15 min. The supernatant, after evaporation to a small volume, was subjected to repeated extractions with chloroform and then the water-phase (*p*H 2–3), saturated with ammonium sulphate, was extracted with secondary butanol. The secondary butanol phase, after adding ethyl ether, was extracted with water. Having thereby removed most of the pigment and impurities, compounds containing cobalt were adsorbed on activated charcoal, washed with 5 per cent phenol, and then eluted with hot 65 per cent acetone. The eluate was evaporated to a small volume to which some non-radioactive concentrated vitamin B_{12} solution was added for a control spot on the electrophoretogram and chromatogram. Throughout the extraction, the *p*H was kept at 2–3 and any cobalamines were maintained in the cyano form by the addition of 0·01 per cent sodium cyanide. Two-dimensional separation of the metabolites was attempted by electrophoresis at a potential of 8 V./cm. for 16 hr. on No. 1 Whatman filter paper in a buffer of 0·5 *N* acetic acid and 0·01 per cent sodium cyanide; followed by descending paper chromatography at right angles to the direction of electrophoresis in a system of water-saturated secondary butanol with 1 per cent acetic acid (w/v) and 0·01 per cent sodium cyanide. The resulting radioactive spots on the chromatogram were resolved by making autoradiographs using X-ray film. The radioactivity in the chromatogram spots was counted in a scintillation counter after elution with water.

Fig. 1 shows the results obtained from a sample of rat bile. The control spot contained only non-radioactive vitamin B_{12}, detected by its colour on the chromatogram, and is represented diagrammatically. The relative amounts of radioactivity contained in

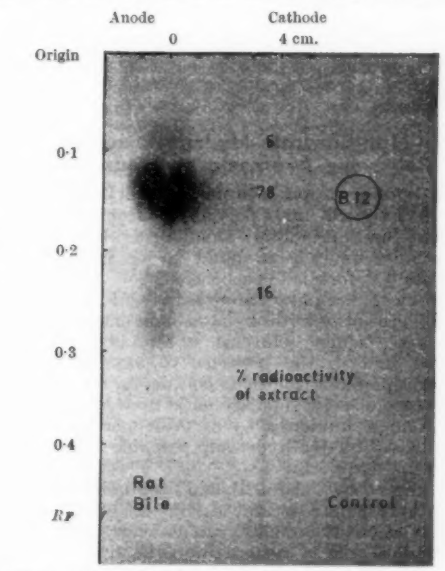

Fig. 1. Autoradiograph of a sample of rat bile following the injection of cobalt-56 vitamin B_{12}

each spot on the chromatogram is shown. A similar result was obtained from a different sample of rat bile.

Comparison with the work of others[2] indicates that the metabolites of vitamin B_{12} here demonstrated in bile are analogues of vitamin B_{12}, and like B_{12} have zero electrophoretic mobility. Free cobalt as cobalt chloride did not migrate in any of the areas shown on the chromatogram. The rat bile contained at least two analogues of vitamin B_{12}. The major portion, approximately three-fourths, of the radioactivity was present as vitamin B_{12}, the remaining fourth being in the B_{12} analogues.

It has been shown by others (see ref. 3) that cyanocobalamine is more readily absorbed in man than the other cobalamines and structural analogues of B_{12}, absorption of the latter being dependent on a strict chemical specificity of the B_{12} benzimidazole group[4]. It seems likely, therefore, that the metabolic products of B_{12} will not be absorbed, or will be poorly re-absorbed once they are excreted in the bile. For this reason, the presence of vitamin B_{12} analogues in bile offers a possible explanation of the appreciable amount of radioactivity sometimes found in the fæces of normal human subjects in the fæcal excretion test of B_{12} absorption[5]. Similarly, it offers an explanation of the finding[1] that re-absorption by the enterohepatic circulation of the radioactivity in human bile after parenteral cobalt-56 vitamin B_{12} was incomplete, being approximately two-thirds of the total radioactivity present.

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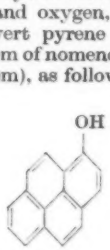
Non-Enzymic Hydroxylation of Polycyclic Aromatic Hydrocarbons

The hydroxylation of aromatic compounds *in vivo* is well known[1], and polycyclic aromatic hydrocarbons[3] and aromatic amino-acids and related compounds[2] have been found to be hydroxylated by suitably fortified suspensions of rodent liver microsomes. Aromatic amino-acids and related compounds are also hydroxylated non-enzymically in phosphate buffer solutions containing iron and ascorbic acid in the presence of oxygen[4,5], or in hydrogen peroxide solutions containing Fe^{++} (ref. 6). In the case of polycyclic aromatic hydrocarbons slow conversion to quinones, by ascorbic acid[7], and more rapid oxidation by hydrogen peroxide[8], have been reported.

Udenfriend *et al.* claimed[4], and Dalgliesh confirmed[5], that the phenols produced by the non-enzymic hydroxylation of aromatic amino-acids, and related compounds, with a system involving iron, ascorbic acid and oxygen, were identical with the phenols produced during animal metabolism. In view of this and the possible connexion between *in vivo* metabolism of polycyclic aromatic hydrocarbons and carcinogenesis[9] the effect of the Udenfriend system on these compounds was examined. It was found (see Table 1) that a system involving Fe^{++}, a suitable complexing agent and oxygen, in a buffer solution, would readily convert pyrene to 3-hydroxypyrene (3 on Richter's system of nomenclature, but 1-hydroxy on Patterson's system), as follows :



3-Hydroxypyrene

Table 1. Conditions for the Hydroxylation of Pyrene

Reaction system	Yield of phenol (mean of five experiments)
Complete system*	38γ
,, minus Fe^{++}	0
,, ,, EDTA	Trace (< 3γ)
,, ,, Ascorbic acid	28γ
,, ,, Fe^{++} and ascorbic acid	0
,, ,, EDTA and ascorbic acid	Trace (< 3γ)
,, ,, Fe^{++} and EDTA	0
,, but with nitrogen instead of oxygen	0
,, but with acetate buffer instead of phosphate	125γ
,, but with Fe^{+++} instead of Fe^{++}	Trace (< 3γ)

* A solution of 500 mgm. L-ascorbic acid, 250 mgm. ferrous sulphate and 500 mgm. of the disodium salt of ethylenediaminetetraacetic acid (EDTA) in 50 ml. of 0·1 *M* phosphate buffer *p*H 5·8 (or 0·1 *M* acetate buffer *p*H 5·8). Pyrene (2 mgm. in 0·4 ml. of ethanol) was added and oxygen gas bubbled through. The reaction was carried out at a temperature of 95° C. for 1 hr.

Ascorbic acid stimulated the reaction, but Fe^{+++} would not replace Fe^{++} even in the presence of ascorbic acid. This is in contrast to the Udenfriend system and with Mazelis's non-enzymic decarboxylation reaction[10]. Replacement of the phosphate buffer by an acetate buffer led to a three-fold improvement in yield. In the experiment at a lower temperature (38° C.) with the complete system, 8 μgm. of 3-hydroxypyrene were obtained from a reaction lasting 1 hr. With solutions containing the complete reaction system but with the Fe^{++} replaced by Cu^{+}, Cu^{++}, Co^{++}, Ce^{+++}, Mg^{++}, Mn^{++} Hg_2^{++} and Fe^{+++} (the valence state of the metal on introduction into the system is specified) either no, or only a trace of, 3-hydroxypyrene was formed.

The activity of the system was dependent on the choice of complexing agent, as shown in Table 2. The failure of cyanide to inhibit this system is in contrast with the behaviour of Warburg's model enzymes[11], based on iron-containing charcoals and the heavy-metal catalysed 'autoxidation' of ascorbic acid[12]. The effect of the complexing agent is undoubtedly to modify the oxidation-reduction potential

Table 2. Effect of Complexing Agents on the Hydroxylation of Pyrene

Reaction system	Yield of phenol
Complete system	
,, plus potassium cyanide*	64γ
,, ,, riboflavin	55γ
,, ,, dipyridyl	Trace (< 3γ)
,, ,, *o*-phenanthroline	0
Complete system minus EDTA	
,, plus sodium citrate	26γ
,, ,, dipyridyl	Trace (?)
,, ,, *o*-phenanthroline	Trace (?)
Fe^{++} and riboflavin only	34γ

* 500 mgm. of each complexing agent was added, but a considerable part of the riboflavin failed to dissolve.

of the iron[13], and it is perhaps not without significance that both αα-dipyridyl and *o*-phenanthroline stabilize strongly the ferrous state[14]. Inhibition of the system by these two agents may, however, be due to hydroxylation of the complexing agent. The hydroxylating power of a combination of Fe^{++} and riboflavin must be of considerable biological interest.

3-hydroxypyrene is the phenol produced by the metabolism of pyrene[15] and the 3 position of pyrene is a site of free-radical attack[16]. It has been found during these experiments that naphthacene, chrysene, 1:2-benzanthracene and 3:4-benzopyrene also react under these conditions and work is now in progress to identify these products.

The 3-hydroxypyrene was identified by extracting the cooled reaction mixture with 3 × 50 ml. of ether, and chromatographing the dried ethereal extracts on alumina. On development with ether, any phenols present passed slowly down the column as bands fluorescing under ultra-violet illumination. Pyrene gave rise to a single bright-blue fluorescent band which after collection was evaporated to dryness under reduced pressure, and redissolved in spectroscopic ethanol. Its absorption spectrum (220–420 mμ) and that of its sodium salt proved identical with those of 3-hydroxypyrene and its sodium salt[17]. The yield was calculated from the absorption at 386 mμ and 366 mμ. Small losses occurred on the chromatography columns, and yields of less than 3γ could not be estimated satisfactorily.

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Kinetics of the Enzymic Degradation of Deoxyribonucleic Acid into Sub-units

Recent reports from this laboratory[1,2] described the enzymic degradation of deoxyribonucleic acid (DNA) into rod-like sub-units having a molecular weight of 5–6 × 10^5 and a Watson and Crick[3] double helical structure. No production of dialysable nucleotides and no increase of the ultra-violet absorption coefficient at 260 mμ were detectable during the digestion. It is the purpose of this communication to report further information obtained by using a purified enzyme preparation.

The crude enzymic preparation from chicken erythrocytes already described[1,2] was purified several hundred times according to a procedure which will be reported elsewhere[4], and used at activity concentrations about 100 times higher than those of the crude preparation used before. Degradation of chicken erythrocyte DNA (which was exclusively used in this work) by the more active enzyme was characterized by: (1) a first phase in which the molecular weight of DNA (as determined by light scattering[5]) dropped rapidly from its starting value of about 6 × 10^6 to about 5–6 × 10^5 (this phase showed all the features already described for the degradation of DNA by the crude enzymic preparation); (2) a second phase in which the molecular weight continued to drop at a slower rate. In this phase, which had not been detected when using the crude enzyme, there was formation of acid-soluble nucleotides and an increase in the ultra-violet absorption coefficient at 260 mμ. A calf thymus acid deoxyribonuclease (DNase) preparation, to be described elsewhere[6], degraded DNA in much the same way as the purified chicken erythrocytes preparation and was used almost exclusively in further work.

As our previous results[1,2] strongly suggested, a non-random breakdown of DNA by the enzyme, and as a similar suggestion had been put forward by Oth *et al.*[7] in their work on the degradation of DNA by acid DNase, the kinetics of the enzymic degradation was investigated in detail. Digestions were carried out in the light-scattering cell at 20°; to 30 ml. of 5–10 mgm./100 ml. of DNA (molecular weight = 6 × 10^6) in acetate buffer pH = 5·4, μ = 0·15, 0·02 ml. of the enzymic preparation (generally containing 10γ protein) was added and molecular weights were determined at intervals of time. No time-lag was detectable, and the initial slope of log $(1 - R)$ versus log t ($R = M_w/M_w^\circ$; M_w = weight average molecular weight; M_w° = initial molecular weight; t = time) was near to 1. Therefore, making the reasonable assumption that $p_t = kt$ (p_t being the probability that any given bond will be cleaved during time t, and k a constant) for values of R between 1 and 0·8, it is concluded that the degradation occurs according to a 'single strand' degradation kinetics[8]. The same behaviour was displayed by DNA samples obtained by enzymic action and ranging in molecular weight from 6 to 1·1 × 10^6.

The degradation of DNA 'sub-units' was studied next. Several samples, obtained by enzymic action from different DNA preparations, and exhibiting molecular weights between 7 and 9 × 10^5 (in acetate buffer pH = 5·4, μ = 0·15), were deproteinized by shaking with chloroform *iso*amyl alcohol and then digested in the light-scattering cells as before (DNA concentrations were 10–20 mgm./100 ml.). A time-lag was evident and the initial slope of log $(1 - R)$ versus log t was close to 2. Therefore, making the same assumptions as before, degradation occurred according to a 'double strand' kinetics.

Finally, the transition phase between the two degradation kinetics was investigated; as expected, this was characterized by a plateau region, in which the molecular weight did not change.

These results show that two different types of degradation are going on at the same time. The 'single strand' degradation is the only one apparent in the first phase, because it is acting during the time-lag of the 'double strand' degradation. Whereas the latter

is a random degradation of the type already known for neutral DNase[8,9], the 'single strand' degradation as shown by this work is highly specific and unequivocally shows that (1) sites exist in DNA ($M_w = 6 \times 10^6$) where the enzymic action brings about the splitting of the molecule without any time-lag and (2) the number of these sites is discrete.

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Relative Sweetness of α- and β-Forms of Selected Sugars

THE mechanism of taste perception has been approached through work on the relationships between chemical properties of taste stimuli and their resultant taste responses. Ferguson and Lawrence[1,2] reported that the D-configurations of leucine, *iso*-leucine, valine, histidine, tryptophan and asparagine were sweet, whereas the L-forms were not. These authors observed that *iso*maltose (6-α-D-glucopyranosyl-D-glucose) was sweet, but its anomer, gentiobiose (6 - β - D - glucopyranosyl - D - glucose), was bitter. Cameron[3,4] found that a freshly prepared solution of 10 per cent α-D-glucose was definitely sweeter than a 16-hr.-old 10 per cent solution. The magnitude of the response (fourteen evaluations) suggested that the fresh solution had a sweetness equal to or more than that of a 10·5 per cent equilibrium solution.

The investigation discussed here was designed to repeat Cameron's work and to determine whether the α- and β-forms of other sugars differ in relative sweetness. The mutarotation-rates at room temperature of glucose, fructose, galactose, and lactose were determined by polarimetric measurements. Equilibrium between the α- and β-forms of these sugars was attained within 2·5 hr. after hydration. Consequently, to ensure having an equilibrium mixture, sugars were hydrated 3 hr. before tasting. Before this experiment, it was established that a freshly hydrated solution of sucrose, which does not undergo mutarotation, did not differ in sweetness from a 16-hr.-old solution.

The taste panel consisted of two women and three men, selected on the basis of their high acuity for detecting sweetness differences from an original group of eleven experienced tasters. Evaluations were made daily at 11.30 a.m. in individual, partitioned booths maintained at a controlled temperature. At each session every judge received four pairs of solutions, one of each pair having been hydrated 3 hr. previously, the other hydrated 100–200 sec. before tasting. The time between hydration of the fresh sample and tasting was kept to a minimum by adding freshly distilled water to the weighed compounds in individual volumetric flasks as each judge entered the booth. Judges were requested to circle the number of the sample within each pair which had the greater sweetness. The order of sample presentation was randomized. Distilled water was provided for oral rinsing at the judge's discretion. A minimum of forty separate evaluations per pair was collected. The method in ref. 5 was used to establish significant differences.

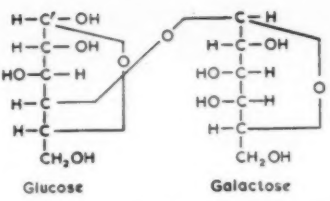

Fig. 1. α-Lactose (D-glucose-4(β-D-galactopyranoside))

As shown in Table 1, the differences in sweetness between the α- and β-configurations became greater as the concentration of the sugar increased. The α-configurations of fructose, glucose and galactose were sweeter than the β-forms, whereas the reverse was true for lactose. This observation is of interest, since the same asymmetric carbon atom (C–1) is responsible for the specific rotation of the lactose molecule as for the glucose molecule (Fig. 1). Rogers[6] suggested that the greater solubility of β-lactose (55·20°C. in 100 parts water) as compared with the α-form (8·90°C.)[7] contributed to its greater sweetness. However, β-D-glucose is more soluble but less sweet than α-D-glucose (154·15°C., 82·25°C., respectively)[6]. When 10 per cent α-D-glucose was used as a sweetener in peach nectar, 76 per cent of 66 comparisons showed

Table 1. RELATIVE SWEETNESS OF α- AND β-FORMS OF FOUR SUGARS

Compound	Concentration (per cent)	No. of evaluations	Sec. after hydration	Response considering (per cent)		
				Alpha sweeter	Equilibrium sweeter	Beta sweeter
α-D-fructose[1]	0·09	40	101	65·0*	35·0	
	0·20	48	103	87·5‡	12·5	
	0·30	40	124	85·0‡	15·0	
	5·00	40	120	90·0‡	10·0	
	7·00	48	126	68·8*	31·2	
α-D-glucose[2]	0·30	40	121	50·0	50·0	
	5·00	40	107	77·5‡	22·5	
	10·00	44	137	88·6‡	11·4	
β-D-glucose[2]	5·00	40	121		75·0†	25·0
	10·00	40	127		85·0‡	15·0
α-versus β-glucose	5·00	40	135	95·0‡		5·0
	10·00	40	148	100·0‡		0·0
α-lactose[4]	0·30	40	117	70·0†	30·0	
	5·00	40	270	40·0	60·0	
	7·00	44	222	13·6	86·4‡	
β-lactose[3]	0·30	48	99		72·9†	27·1
	5·00	40	124		45·0	55·0
	7·00	40	140		35·0	65·0*
α- versus β-lactose	5·00	40	184	25·0		75·0†
	7·00	40	266	12·5		87·5‡
α-D-galactose[2]	5·00	40	130	62·5	37·5	
	10·00	40	185	72·5†	27·5	

* Significant at $P = 0.05$. † Significant at $P = 0.01$. ‡ Significant at $P = 0.001$.

[1] Eastman Organic Chemicals, Lot 679. [2] Eastman Organic Chemicals, Lot 84. [3] Nutritional Biochemicals Corp. [4] Eastman Organic Chemicals, Lot 357.

the freshly sweetened nectar to be sweeter than that sweetened 6 hr. before.

In extensive work on the effectiveness of various carbohydrates in stimulating labellar and tarsal hairs of the blowfly, *Phormia regina*, Dethier[9] observed no significant differences in thresholds between α-D-glucose and an equilibrium mixture. On the other hand, Dethier demonstrated a difference between α-nitroglucose and β-nitroglucose, the former being somewhat more effective. The most striking difference was found in the great effectiveness of α-methyl glucoside in contrast to the ineffectiveness of β-methyl glucoside. The most effective carbohydrate stimuli for *Phormia regina* included compound sugars with an α-D-glucopyranoside link. Enzyme inhibitors such as azide, iodoacetate, phlorizin and fluoride did not prevent stimulation.

In his review on the relationship between molecular structure and odour, Stoll[10] stated that if optical isomers are distinguishable by their odour, molecules constituting the receptors may be optically active also, and thus exhibit a different adsorbent activity towards the odorous molecules in contact with them according to the sign of rotation of the latter. Lawrence and Ferguson[2] tested the theory that the stereospecific surface of the taste organ might be that of an enzyme, by measuring the adsorption of sweet and non-sweet isomeric pairs of compounds on charcoal, cut human hair, and tongue surfaces. No significant differences in adsorption were observed. In addition, these investigators found no obliteration of the sweet taste in the presence of the enzyme inhibitors, sodium azide, potassium fluoride, sodium iodoacetate, and sodium cyanide. Baradi and Bourne[11,12] reported that in the papilla foliata of the rabbit, quinine inhibited glycerophosphatase, 5-nucleotidase and esterase reactions, and greatly increased the ribonuclease reaction. Sucrose had no effect and saccharine inhibited esterase.

It must be concluded that the reason for differences in sweetness evoked by anomers of various compounds remains to be elucidated. The results reported here confirm Cameron's observations on glucose[3] and establish that the anomers of fructose, galactose, and lactose differ in intensity of sweetness also. Consequently, to assure equilibration of the α- and β-forms, sweetness investigations of asymmetrical sugars should involve hydration several hours before tasting.

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BIOPHYSICS

The Action Potential in Cells of *Chara*

In the resting state of giant internodal cells of the fresh water algæ, *Chara* and *Nitella*, an electric potential difference exists between the interior of the cells and an external medium. The value of this potential difference appears to be determined by the permeability of the plasmalemma (outer cytoplasmic membrane) to potassium ions and, to a lesser extent, to sodium ions[1,2]. The resting resistance of this membrane is also related to the permeability to these ions and to their external concentrations[2].

The action potential, which is a reversible depolarization of the plasmalemma[3], occurs on stimulation of the cells by a pulse of current or by abrupt change in temperature, etc. It might be suggested that the action potential in the Characeae, which is qualitatively similar to the conducted impulse in a nerve axon, is similarly related to quick changes in permeability of the cell.

Osterhout[4] proposed a mechanism involving potassium ions and the inner cytoplasmic membrane (tonoplast), but this seems unlikely since, in fact, the action potential occurs across the plasmalemma and the potassium ion is almost in electrochemical equilibrium across it. Thus the potential difference would not be expected to change greatly if a change towards increased permeability to potassium occurred.

Gaffey and Mullins[5] reported that increased fluxes of potassium and chloride accompanied the action potential in cells of the corticated species *Chara globularis* and that the potential difference at the peak of the action potential was influenced by the concentration of chloride in the external medium.

The present investigation is concerned with the effects of changes in concentration of various ions on the action potential in single cells of *Chara australis*. An alternative explanation of some of the results of Gaffey and Mullins is offered.

Continuous records on a chart recorder were made of the potential difference between a microelectrode in the vacuole and an external medium. A second electrode in the vacuole was used to pass current pulses to initiate action potentials. The threshold was generally about 30 mV. above the resting level. To investigate the effect of changes in concentration of a particular ion, the total concentratien was kept constant and the concentration of one ion species varied at the expense of another, so that the cell wall potential remained constant[3].

Fig. 1 shows the resting potential difference and the potential difference reached at the peak of the action potential, as functions of calcium concentration, $[Ca^{++}]_o$, in solutions of calcium and magnesium chloride of total concentration 6 m*N*. All solutions also contained 1 m*N* sodium chloride and 0·1 m*N* potassium chloride. While the resting potential was almost unaffected by changes in concentration of calcium or magnesium, the peak of the action potential was changed reversibly, a new value being reached within about 10 min. of changing the concentration. It is seen that the peak of the action potential approximately follows a line with a slope of 29 mV./$(10 \times [Ca^{++}]_o)$, but with a tendency (observed in several experiments) for the peak to be more negative than the extrapolation of this line at low calcium concentration.

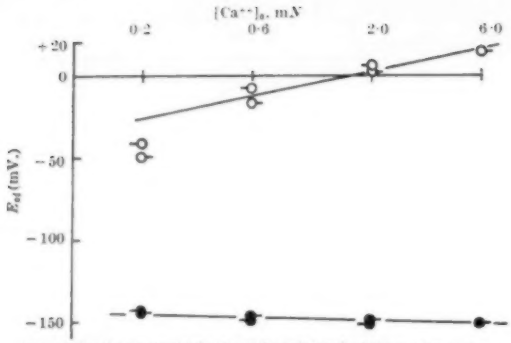

Fig. 1. Resting potential (●) and peak level of the action potential (○), plotted against calcium concentration on a logarithmic scale

Bars on the symbols show direction of increasing or decreasing concentration, starting and finishing at 0·2 mN. The line through some of the points for action potential has a slope of 29 mV. per ten-fold change in external calcium concentration

In the presence of calcium ions, changes in concentration of other ion species had no consistent effect on the peak of the action potential. In the absence of added calcium ions, the peak did not hold a steady value, but became more negative with time as a consequence of the removal by cation exchange via the cell wall Donnan phase[2] of the calcium in the immediate neighbourhood of the plasmalemma. Fig. 2 shows the decline in the magnitude ΔE of the action potential (the change in potential from resting-level to peak) in solutions of choline chloride. The rate of decrease of ΔE was greater in 10 mN than in 3·3 mN choline chloride. This agrees qualitatively with comparative rates of exchange in isolated cell walls of *Chara*[6].

In solutions not containing calcium, the cell eventually reached a stage where it could not be stimulated by pulses depolarizing the cell by as much as 100 mV.

It is now suggested that, had the changes in the experiments of Gaffey and Mullins[5] been made in the direction of increasing choline chloride concentration, the magnitude of the action potential would have appeared to decrease with increasing concentration of chloride (Fig. 3 in Gaffey and Mullins[5]), whereas in fact the decrease was a function of time. However, the reported extra fluxes of chloride during the action potential in *Chara globularis* remain unexplained.

In the present experiments the action potential may be described in terms of a sudden increased permeability of the plasmalemma to calcium such that the potential approaches the ion equilibrium potential for this ion, given by:

$$E_{oi} = \frac{RT}{2F} \ln ([Ca^{++}]_o/[Ca^{++}]_i) \quad (1)$$

$$= \text{const.} + 29 \log_{10} [Ca^{++}]_o \text{ at } 19^\circ \text{ C.} \quad (2)$$

The effect of calcium was obtained in the presence of concomitant changes in magnesium, barium, or strontium concentration and is therefore specific to calcium. Changes in permeability to other ions besides calcium may be involved in the action potential. This may explain some observed deviations from the relation predicted by equation (1) or (2).

This work is part of the joint programme of the Division of Food Preservation, Commonwealth Scientific and Industrial Research Organization, and the Botany School, University of Sydney.

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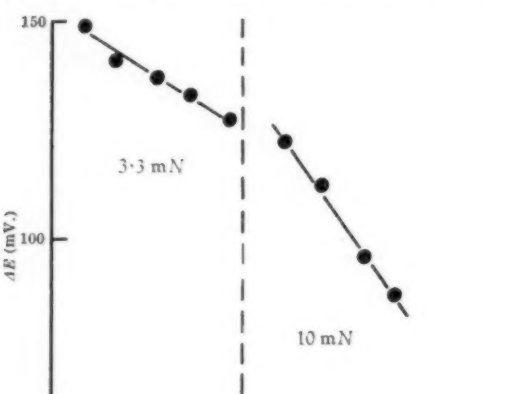

Fig. 2. The magnitude (ΔE) of the action potential in a cell in 3·3 mN and 10 mN choline chloride, plotted against time

The change in concentration of choline chloride was made at the time indicated by the dotted line

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Voltage-Clamp Experiments with *Nitella*

Hope[1] has suggested that in the large internodal cells of *Chara australis*, the depolarization of the plasmalemma during an action potential is caused by a sudden transient increase in the permeability of this membrane to calcium ions, and a consequent inward movement of these ions. This suggestion followed from an investigation of the effects of changes in concentration of various ions on the action potential in single *Chara* cells.

Recently, in this laboratory, it has been possible to examine in more detail the action potential process by applying a 'voltage-clamp' technique to the ecorticate internodal cells of a species of *Nitella*. The action potential in these cells has been described previously by Findlay[2]. In the voltage-clamping method a feed-back circuit is used to change the membrane potential to some pre-determined level and maintain it as close as possible to this level during the electrical activity of the cell. The current flowing across the membrane during the voltage-clamp is recorded. Fig. 1 shows the relationship between membrane current and time, for different membrane potentials. It is seen that the current may be divided into two components: a transient inward current and a steady outward current.

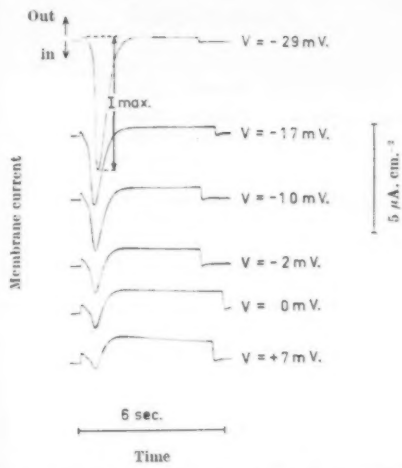

Fig. 1. Records of membrane current under a voltage-clamp. The membrane potential is shown at the right of each curve. The maximum inward current is shown as I_{max}.

Whereas the outward current increases as the membrane potential is changed in the positive direction, the inward current decreases, and eventually becomes zero.

The effect of external calcium concentration on these currents was investigated. The membrane potential – maximum inward current relationship for different concentrations of external calcium, $[Ca^{++}]_{ext.}$, was determined. It was also found that $[Ca^{++}]_{ext.}$ had little effect on the steady, outward-flowing current. Some results are shown in Fig. 2*a*. In this experiment, the cell was bathed in a solution of calcium and magnesium chloride and the calcium concentration varied at the expense of the magnesium. The total ionic concentration of the solution was kept constant throughout the experiment to eliminate changes in potential difference across the cell wall Donnan system[3]. In Fig. 2*b* the membrane potential for which the inward current was zero and the potential at the peak of an ordinary action potential are plotted against $[Ca^{++}]_{ext.}$

The role of the chloride in the action process was examined by determining the relationship between the clamped potential (V) and the maximum inward current ($I_{max.}$) for different $[Cl^-]_{ext.}$ and fixed $[Ca^{++}]_{ext.}$. The results are shown in Fig. 3. Curves (*a*) were obtained with $[Ca^{++}]_{ext.}$ = 0·1 mN and $[Cl^-]_{ext.}$ = 0·1, 0·3, 1·0 mN, and curves (*b*) with $[Ca^{++}]_{ext.}$ = 1·0 mN. The three curves in each of the two groups lie close to one another, and intersect the membrane potential axis at approximately the same position.

The results summarized in Fig. 2 show that the membrane potential for which the initial inward current is zero depends on $[Ca^{++}]_{ext.}$. The slope of the $V/I_{max.}$ curve, a measure of the minimum resistance of the membrane during activity, is of the order of 2–4 k.Ωcm.², while the resting resistance of the *Nitella* membrane is 30–80 k.Ωcm.². Fig. 3 shows that $[Cl^-]_{ext.}$, however, has little effect on the $V/I_{max.}$ relationship. These results indicate that the plasmalemma of *Nitella* during the initial stage of the action potential undergoes a transient increase in permeability to calcium. During this time calcium controls the $V/I_{max.}$ relationship. Hence, the membrane potential for which the calcium current is zero is the electrochemical equilibrium potential for calcium. Consequently, if $[Ca^{++}]_{ext.}$ is changed the intercept of the $V/I_{max.}$ graph with the V axis should vary according to the Nernst equation, namely a change of 29 mV. for each ten-fold change in $[Ca^{++}]_{ext.}$. However, it is found that in *Nitella* the change is usually 35–40 mV. One possible explanation of this larger value is that calcium is partly in the form of a monovalent complex, and both this and the divalent calcium together control the action potential. The 35–40 mV. change for ten-fold change in $[Ca^{++}]_{ext.}$ would represent the combined behaviour of the two forms of calcium. A plot of the potential at the top of the

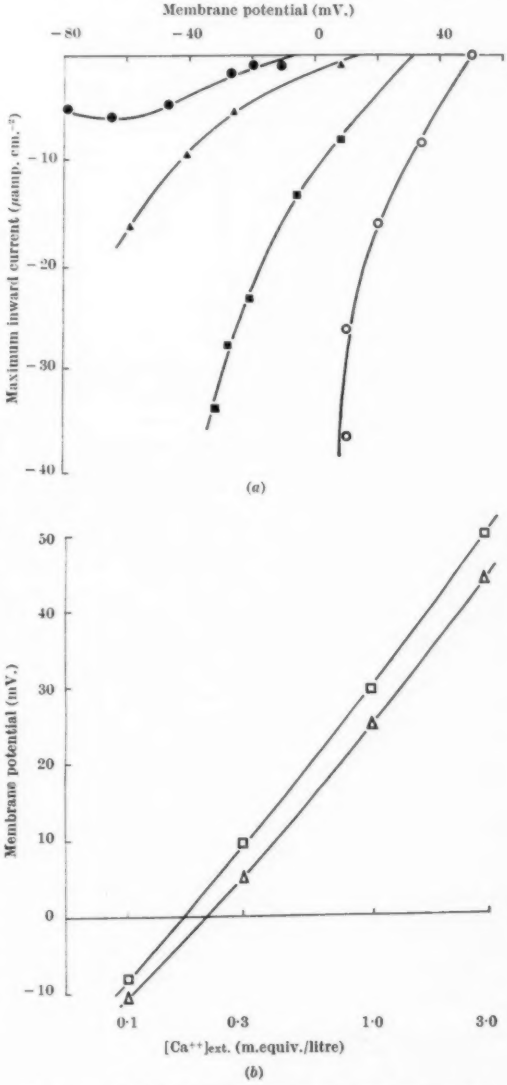

Fig. 2. (*a*) Relationship between maximum inward current and membrane potential for different $[Ca^{++}]_{ext.}$ ●, 0·1 mN; ▲, 0·3 mN; ■, 1·0 mN; ○, 3·0 mN. (*b*) Relationship between $[Ca^{++}]_{ext.}$ and membrane potential for $I_{max.}$ = 0 (□), and potential at peak of ordinary action potential (△)

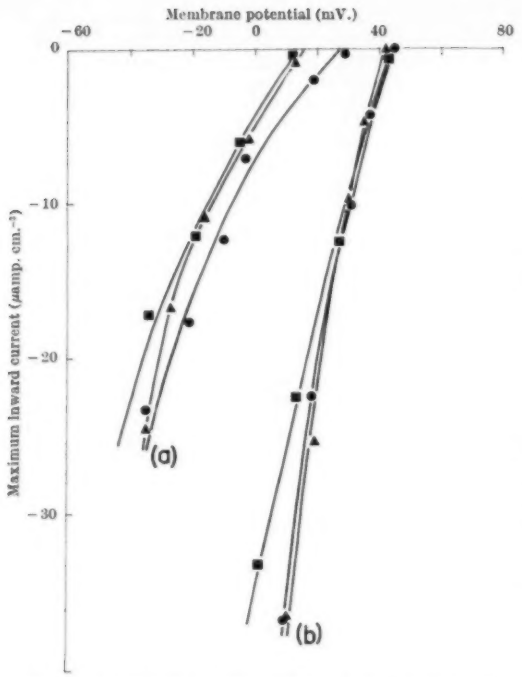

Fig. 3. Relationship between maximum inward current and membrane potential for varying $[Cl^-]_{ext.}$ and $[Ca^{++}]_{ext.}$ Curves (*a*) 0·1 m*N*; curves (*b*) 1·0 m*N* $[Ca^{++}]_{ext.}$ with $[Cl^-]_{ext.}$ = 0·1 (●); 0·3 (▲), and 1·0 (■) m*N*

spike of an ordinary action potential against $[Ca^{++}]_{ext.}$ also has a slope of 35–40 mV. per tenfold change in $[Ca^{++}]_{ext.}$. Furthermore, it is possible, as Hope[1] suggests, that other ions besides calcium may be involved to some extent in the action potential, and that the peak potential is not the electrochemical equilibrium potential for calcium alone.

Further evidence for an increase in membrane permeability to calcium during activity is afforded by the result that for any one membrane potential-level the maximum inward current increases as $[Ca^{++}]_{ext.}$ increases. This is not the case with chloride.

The foregoing experiments thus substantiate Hope's[1] conclusion that the calcium ion plays a major part in the action potential process. The voltage-clamp results show that the action potential (initiated when the membrane potential is raised above the threshold) results from a transient increase in membrane permeability to calcium. The membrane potential changes towards the electrochemical equilibrium potential for calcium. However, before this potential-level is reached (see Fig. 2*b*), the calcium permeability has decreased, and the membrane potential returns to the resting-level. The recovery is probably the result of an outward movement of potassium ions.

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PHYSIOLOGY

Spontaneous Miniature Potentials from Insect Muscle Fibres

Recent reports[1,2] describe spontaneous potential changes at crustacean neuromuscular junctions which bear a close resemblance to vertebrate miniature end-plate potentials[3]. Quantal 'packets' of acetylcholine, released from the pre-synaptic terminals of the vertebrate motor nerve, produce transient depolarizations at the post-synaptic muscle membrane of about 0·5 mV. Since reports of a similar type of activity at the insect neuromuscular junction have so far been lacking, it appeared of interest to submit the following account of its occurrence in this group.

Randomly occurring spontaneous potentials of up to 1 mV. have been recorded, in the giant South American cockroach, *Blaberus giganteus*, from single muscle fibres of the extensor tibiæ using internal micro-electrodes of resistance between 10 and 20 M.ohm. Considerable variations were observed (Fig. 1) in both amplitudes and rise times. These findings are consistent with the multi-terminal nerve innervation known to be present in insects[4,5]. The similarity of these transients to the spontaneous phenomena exhibited by the 'slow' fibres of the frog[6] is very evident. It is possible to visualize a series of junctions displaced along the muscle fibre, discharging randomly to produce a variety of potential types at the recording site.

Values for discharge frequencies collected from fibres of a large number of specimens followed a normal distribution curve ranging from 1 per sec. to 15 per sec. In general, the values were rather higher than those recorded for either the frog or the crayfish, and it is possible that the saline used[7] was slightly hypertonic to the body fluids. An increase in tonicity leads to a rise in the rate of discharge. For example, when the normal saline was replaced with one made five times hypertonic with sucrose, a marked increase in the frequency occurred over the first minute after application, followed by a gradual decline to zero over the next 30 min. Following return of the preparation to fresh normal saline, the normal discharge frequency returned (Fig. 2).

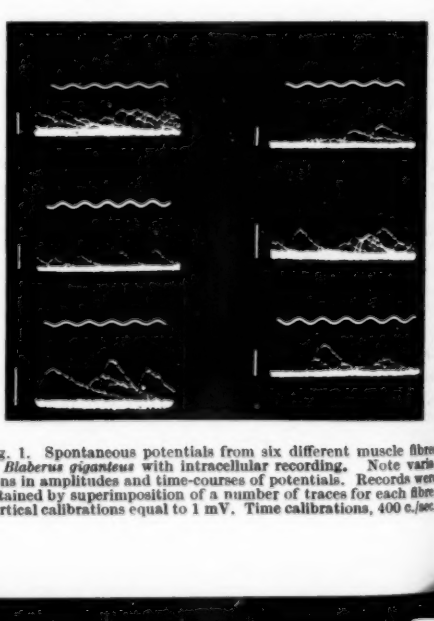

Fig. 1. Spontaneous potentials from six different muscle fibres of *Blaberus giganteus* with intracellular recording. Note variations in amplitudes and time-courses of potentials. Records were obtained by superimposition of a number of traces for each fibre. Vertical calibrations equal to 1 mV. Time calibrations, 400 c./sec.

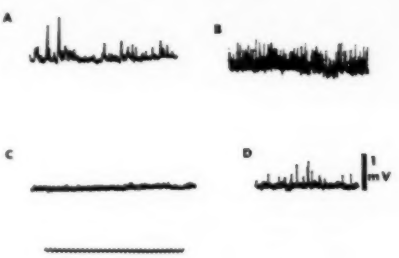

Fig. 2. Effect of tonicity on amplitude and frequency of spontaneous potentials. (*A*) Normal saline; (*B*) effect after 1 min. of increasing tonicity by a factor of five through the addition of sucrose to the saline; (*C*) effect after 30 min.; (*D*) return to normal tonicity. Voltage calibration same for *A*, *B*, *C* and *D*. Time calibration same for *A*, *B*, *C* and *D* at 30 c./sec.

A few recordings from the metathoracic extensor tibiae muscle fibres of the locust *Schistocerca gregaria* followed closely the pattern described for *Blaberus giganteus*.

It seems possible that the quantal transmitter discharge characteristic of the vertebrate nerve ending is also to be found in the insects, although the chemical identity of the transmitter substance is not yet known for any insect neuromuscular junctions.

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Thermodynamics and Osmoregulation

During a recent meeting concerning renal function one of the speakers suggested that the reason for the high metabolic rate of the mammalian kidney might be further investigated since very little energy seems to be used in forming the urine. The allusion was to a computation by Borsook and Winegarten[1] showing that the thermodynamic efficiency of the kidney is about 1 per cent. However, the inference, encountered not infrequently, and not only in connexion with renal function, is based on a misunderstanding of the nature of the computation.

The thermodynamic work performed by an organ like the kidney is the sum of a sequence of terms $RT \ln \frac{[a]\ \text{blood}}{[a]\ \text{urine}}$ for each of the components of the urine. Here $[a]$ is the activity of the substance, usually approximated by its concentration. It is important to note that this value depends solely on the initial and final states of the system (for example, blood and urine concentrations of the constituents), and on the temperature. It is completely independent of the mechanism by which the urine is formed. The actual energy expended by an organ like the kidney is a function of these mechanisms, and it is this parameter, and not the thermodynamic work done, that is reflected in the oxygen consumption of the organ. To return to the case of the mammalian kidney, if we take account of the manner in which the urine is formed, and use some recently published results on the energetics of sodium transport, it is easy to show that there is no mystery about the high oxygen requirement of this organ. It has been shown that about 16 equivalents of sodium ion are transported across the frog skin per mole of oxygen used[2,3]. In the course of a day the human kidneys filter about 30 equivalents of sodium, nearly all of which is re-absorbed. If we assume that all the re-absorption requires energy, and use Zerahn's value for sodium/oxygen (thereby assuming that the renal 'sodium pump' is the same as that in the skin of the frog) the rate of oxygen consumption would be about 25 ml./min. This is actually slightly higher than the total oxygen consumption for the organs, and no account has been taken of energy expended in re-absorbing other solutes. It is possible that some sodium is re-absorbed by systems with a lower energy requirement (for example, by forced exchange against H^+ and NH_4^+), or that sodium/oxygen < 16. The reasonable correspondence between observed and computed values, however, makes it obvious that a large fraction of the energy turnover must be devoted to solute re-absorption.

This type of misconception is not restricted to discussions on the mammalian kidney. Schlieper[4] has summarized much evidence showing that the oxygen consumption of aquatic organisms increases as their environment becomes more dilute. These data have been interpreted to mean that increased work is being done by the osmoregulatory organs in dilute media. This interpretation has been criticized, and one of the major objections has been based on the same misuse of the thermodynamic argument. Potts[5] has shown that the thermodynamic work done by the gills and kidneys of these organisms represents a very small proportion of their total energy turnover. Potts's data have been used as the basis for the argument that increased activity of the osmoregulatory organs could not account for the rise in oxygen consumption, which is often as large as 20–40 per cent[6,7]. As in the previous case the computation concerns thermodynamic work and is a minimal, not a realized value. The actual energy expended by the osmoregulatory organs depends on the mode of their operation; not on the initial and final states, but on the path by which the transformation is made. Thus, oxygen consumption of the crayfish antennal gland is about 5 per cent of the animal's total[8], certainly not a negligible fraction. If this organ, like the vertebrate kidney, functions on a filtration–re-absorption basis[9], it is likely that cessation of urine formation alone would cause a measurable fall in basal oxygen utilization. Such a cessation has been noted when the osmotic concentration of the external environment is raised. In addition, blood sodium rises when the concentration in the environment is increased. Since the rate of sodium uptake by the gills varies inversely with blood sodium-level[11], energy expenditure by the gills might also be expected to decrease in brackish water. The result would be just that noted by Schlieper: lower energy expenditure in the more concentrated medium. Thus, if the mechanisms are considered, an appreciable change in resting oxygen consumption is not only reasonable, but might have been predicted. However, it is not the chief purpose of this communication to defend Schlieper's thesis, but to point out that the thermodynamic argument against it is not valid.

While the thermodynamic work performed sets no upper limit on the amount of energy that may be expended it does provide an absolute minimum. Thus it is useful in evaluating models that are proposed for solute and solvent transport. In addition, use of a thermodynamic approach to osmoregulation has led to some interesting speculation about the evolution of these systems. If this approach is to be fruitful, however, the limitations of thermodynamics have to be observed.

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Effects of Drugs on Uptake of Isotopic Norepinephrine by Cat Tissues

Previous experiments have shown that catecholamines enter tissue slices both by simple diffusion and by a concentrating mechanism that can be demonstrated in brain, heart and spleen, but not in muscle or liver, when these tissues are incubated with physiological concentrations of isotopic epinephrine or norepinephrine[1,2]. The uptake by the concentrating mechanism is maximal in media containing 10–25 mμgm. of norepinephrine per ml. and is inhibited by reserpine and ouabain. At concentrations of norepinephrine greater than 200 mμgm. per ml., uptake by the concentrating mechanism becomes negligible in comparison to the amount entering by diffusion and the effect of reserpine is no longer demonstrable.

The present experiments were undertaken in the hope that the effects of several drugs on the uptake of norepinephrine may be related to their pharmacological effects. Slices of cat tissue were incubated for 45 min. in Krebs bicarbonate medium containing the appropriate drug and 5 mμgm. of *dl*-norepinephrine-7^3H per ml. The concentration of radioactivity in control slices of cortex, heart or spleen incubated without drug was usually about four times that in the medium. Approximately 80 per cent of the total radioactivity in the slices after incubation was accounted for chromatographically[3] as unchanged norepinephrine. The effects of a number of drugs on the uptake by the concentrating mechanism are listed in Table 1. In these experiments diffusion alone may be expected to give concentrations of norepinephrine in tissue approximately equal to that in the medium[2]. Percentage inhibition of the concentrating mechanism is therefore expressed as $100 \times (C_C - C_D)/(C_C - C_M)$, where C_C and C_D are the concentrations of isotopic norepinephrine in the tissue of the control and drug-treated slices respectively and C_M is the concentration in the incubation medium.

Table 1. Effect of Drugs on the Concentration of Radioactivity in Slices of Cat Tissue incubated 45 min. with *dl*-Norepinephrine-7^3H (5 mμgm./ml.)

Tissue		Molar concentration	Inhibition of uptake (per cent)
Cortex	Reserpine	10^{-6}	78
	Isoreserpine	10^{-6}	0
	Chlorpromazine	10^{-6}	72
	Cocaine	10^{-6}	100
	Mescaline	10^{-6}	32
	Lysergic acid diethylamide	10^{-5}	0
	Lysergic acid diethylamide	10^{-7}	0
	Lysergic acid diethylamide	10^{-6}	0
	Acetyl choline (with 25 μgm. eserine per ml.)	5×10^{-6}	0
	5-Hydroxytryptamine	5×10^{-6}	0
Spleen	Dibenamine	10^{-6}	0
	Dibenamine	10^{-5}	0
	Dibenamine	10^{-4}	0
	Ergotamine	10^{-6}	30
	Regitine	2×10^{-6}	14
	Chlorpromazine	10^{-6}	65
	Dichloroisoproterenol	10^{-6}	0
	Dichloroisoproterenol	5×10^{-5}	50
	Tyramine	5×10^{-6}	36
	Ephedrine	5×10^{-6}	32
	D-Amphetamine	5×10^{-6}	57
	Cocaine	10^{-6}	100
	Bretylium	3×10^{-5}	73
	Guanethidine	10^{-5}	78
	Tofranil	10^{-6}	77
Heart	Reserpine	10^{-6}	70
	Recanescine	10^{-6}	71
	Rescinnamine	10^{-6}	72
	Isoreserpine	10^{-6}	0
	Cocaine	10^{-6}	100

Norepinephrine uptake in heart slices was used for a comparison of the inhibitory effects of equimolar concentrations of several alkaloids structurally related to reserpine. The sedative agents, reserpine, recanescine and rescinnamine, were effective inhibitors. It is noteworthy that isoreserpine, which is without effect on uptake of norepinephrine, differs from reserpine only in the configuration at one carbon atom.

Both sympathomimetic and sympatholytic drugs are among the substances that inhibit the uptake of norepinephrine. Some of the former, including tyramine, ephedrine and amphetamine[4], may exert their effects by displacing norepinephrine from tissue stores near sympathetic nerve endings, thus making the amine available to receptor sites. The structural similarity of these amines to norepinephrine suggests that the displacement may result from competition by these agents for tissue storage sites.

Other inhibitory agents also release catecholamines from tissues, presumably by other mechanisms. The depletion of catecholamines by reserpine[5–7], for example, may result from some slowly reversible change in cell structure, since the effects of this drug persist for so long a time after its disappearance from the body. Guanethidine is unusual in that it depletes peripheral tissues of norepinephrine, but not of 5-hydroxytryptamine (5HT) (ref. 8). Experiments with blood platelets indicate that depletion of 5HT may result from interference by reserpine with an active transport mechanism that helps to maintain high intracellular levels of the former[9]. Depletion of norepinephrine stores in cells may be related to the effect of reserpine-like drugs on the uptake mechanism reported here.

The property most commonly encountered among these inhibitory drugs is the enhancement of the response of adrenergic organs to norepinephrine administered. This occurs with cocaine[10], reserpine[11], and bretylium[12] as well as with some sympathomimetic amines[5]. Sensitization can be demonstrated with chlorpromazine[13]. Thyroxine, which has been found in a separate investigation to block the uptake of norepinephrine (Dengler, H. J., unpublished results), is also a potentiator.

The uptake mechanism may well be a means for terminating the biological response to catecholamines by removing them from the site of activity. Should this be so, sensitization by these drugs would be expected as a consequence of their inhibitory properties. Evidence for the termination of biological effect by some sort of intracellular re-distribution rather than by metabolic alteration of the catecholamines has been reviewed recently[14].

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Stimulation by Aldosterone of Sodium Transport in the Loop of Henle

Jones and de Wardener[1] have reported that normal subjects, when hydropenic, excrete a urine osmotically more concentrated than when given large quantities of vasopressin while hydrated. During dehydration, one element coming normally into play, besides antidiuretic hormone, is a hypersecretion of aldosterone by the adrenal cortex, as a response to the shrinkage of the liquid compartments of the body[2,3]. The possible influence of aldosterone on the concentrating capacities of the human kidney was therefore evaluated by comparing the urinary osmotic pressure after administration to hydrated subjects of vasopressin alone and of vasopressin associated with aldosterone.

Seven tests were performed on five normal young males. Two and a half units of 'Pitressin' tannate were injected subcutaneously at 8 a.m., first alone, then, one week later, in association with 0·5 mgm. of D-aldosterone-21-monoacetate in oil. The subjects emptied their bladders 2 hr. after the injections, and collected urine thereafter for a period of 8 hr. during which they were asked to drink about 1 litre of fluids in addition to their usual diet. The urine osmolality (m.osm./kgm. water) was determined with a Fiske osmometer.

The results are presented in Table 1. In the presence of aldosterone, there was a larger increase in urine osmotic pressure after injection of vasopressin.

The mean urine osmolality figure of 955 obtained after administration of vasopressin alone is comparable with the mean value of 972 reported by Jones and de Wardener, who indeed pointed out that the dose of 2·5 units of vasopressin sufficed to produce a maximal effect on the urinary osmotic pressure of their hydrated normal subjects.

Table 1. URINE OSMOLALITY IN NORMAL HYDRATED MALES AFTER VASOPRESSIN ASSOCIATED WITH OR WITHOUT ALDOSTERONE

Subjects	Vasopressin		Vasopressin + Aldosterone	
	Urine volume	Osmolality	Urine volume	Osmolality
J. C.	580	849	290	1,093
J. C.	460	1,098	305	1,175
J. C.	415	1,036	315	1,115
K. D.	310	933	350	907
M. V.	450	943	450	986
R. D.	430	885	160	1,024
R. B.	250	944	390	961

Mean $\Delta\pi$: 81·9. *S.E.* $\Delta\pi$: 33·4. $P < 0{\cdot}05$.

If aldosterone improves the concentrating ability of the human kidney, the conclusion may be drawn that this hormone stimulates the re-absorption of sodium (chloride) in the ascending limb of the loop of Henle. Sodium transport at that site, from the tubular lumen to the interstitium, is taken as the prime mover in the operation of the counter-current concentrating mechanism of the renal papilla[4,5]. Factors exerting an effect on the rate of sodium transport at that level would in turn influence the efficiency of the whole process of urine concentration. As suggested by the results presented here, aldosterone is one of these factors. They indicate, furthermore, that, even when stimulated by aldosterone, sodium transport in that part of the nephron is not linked to the secretion into the urine of other cations. It is pertinent to mention here that aldosterone has recently been shown to enhance active sodium transport in the isolated urinary bladder of the toad[6], a preparation in which sodium (chloride) movement is not coupled with cationic secretion[7].

I wish to express my gratitude to Mr. P. Denis, Parke, Davis and Co., for the gift of 'Pitressin' tannate, and to Prof. R. Gross, Ciba A. G., Basle, for the gift of D-aldosterone-21-monoacetate.

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PHARMACOLOGY

Inhibition of Phosphorylation by Atractyloside

In previous papers it was shown that atractyloside, a glycoside (formerly called K-atractylate) extracted from the rhizome of *Atractylis gummifera*, the aglycone of which contains the perhydrophenanthrene nucleus[1], inhibits the uptake of oxygen by rat-liver mitochondria also at very low concentrations (0·0000036 *M*) and with a marked decrease of the P/O ratio[2].

Using hypotonically treated rat-liver mitochondria, with reduced diphosphopyridine nucleotide or ferrocytochrome-c as substrates, inhibition of phosphorylation was observed without decrease in the rate of oxygen uptake[3]. The 2,4-dinitrophenol-induced adenosine triphosphatase of intact mitochondria was inhibited by this drug, but the same was not true for Mg^{++}-activated adenosine triphosphatase of mitochondria disrupted by deoxycholate[4].

In this communication evidence is presented that inhibition of mitochondrial respiration by atractyloside might be due exclusively to interference with phosphorylation reactions.

Table 1 shows that: (*a*) atractyloside has no effect on the oxygen uptake of water pre-treated mitochondria oxidizing β-hydroxybutyrate; (*b*) atractyloside fails to produce inhibition of respiration in an adenosine triphosphate-, glucose- and hexokinase-deficient system and in a phosphorus-deficient system; (*c*) the respiration of mitochondria, observed under phosphorylative conditions in the presence of atractyloside, is very similar to that of mitochondria incubated in the absence of adenosine triphosphate (ATP), glucose and hexokinase; this fact could indicate that the increase of uptake of oxygen due to phosphorylation is inhibited by the drug. Phosphorus was estimated by the Fiske and Subbarow method and a complete block of phosphorus uptake was observed in the vessels containing atractyloside.

Table 1. Effect of Atractyloside on Mitochondrial Respiration
The oxygen uptake was measured manometrically at 26° C. in the Warburg apparatus. The vessels contained: (*a*) 0·03 *M* potassium phosphate buffer, *p*H 7·4, 0·005 *M* $MgSO_4$, 0·00036 *M* diphosphopyridine nucleotide, 0·001 *M* ethylenediamine tetraacetate, 0·09 *M* sucrose, 0·02 *M* β-hydroxybutyrate, 1 mgm. of nitrogen rat-liver mitochondria (isolated in 0·25 *M* sucrose and pre-treated with water for 30 min. at 0° C). (*b*) 0·015 *M* potassium phosphate buffer, *p*H 7·4, 0·03 *M tris* buffer, *p*H 7·4, 0·005 *M* $MgSO_4$, 0·03 *M* glucose, 0·0014 *M* ATP, 0·8 mgm. yeast hexokinase (Sigma type II), 0·01 *M* glutamate (or 0·02 *M* β-hydroxybutyrate), 0·001 *M* ethylenediamine tetraacetate, 0·09 *M* sucrose, 0·000005 *M* cytochrome-*C*, 1 mgm. of nitrogen rat-liver mitochondria (isolated in 0·25 *M* sucrose). Final volume, 2·8 ml.; time of incubation, 20 min.; exceptions are indicated; gas-phase, air; KOH and filter paper in the centre well. Inorganic phosphate was omitted by the Borst and Slater (ref. 5) procedure; P/O ratios are reported in parentheses.

Substrate	Omissions	Oxygen uptake (μatoms) Without atractyloside	Oxygen uptake (μatoms) Atractyloside 0·00005 *M*
(*a*) Water pre-treated mitochondria			
β-Hydroxybutyrate	Nil	5·8	5·8
(*b*) 0·25 *M* sucrose mitochondria			
β-Hydroxybutyrate*	Nil	2·9 (2·6)	1·2 (0)
β-Hydroxybutyrate*	ATP, glucose, hexokinase	1·2	1·2
β-Hydroxybutyrate*	inorganic phosphate	1·1	1·1
Glutamate	Nil	14·1 (2·6)	2·0 (0)
Glutamate	ATP, glucose hexokinase	2·3	2·0
Glutamate	inorganic phosphate	1·4	1·3

* Time of incubation 10 min.

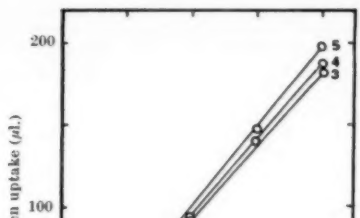

Fig. 1. The effect of atractyloside on the rate of glutamate oxidation under phosphorylative conditions and in presence of 2,4-dinitrophenol. The vessels contained 0·015 *M* potassium phosphate buffer, *p*H 7·4, 0·03 *M tris* buffer, *p*H 7·4, 0·005 *M* $MgSO_4$, 0·001 *M* ethylenediamine tetraacetate, 0·01 *M* glutamate, 0·09 *M* sucrose, 0·000005 *M* cytochrome-*c*, 1 mgm. of nitrogen rat-liver mitochondria (isolated in 0·25 *M* sucrose). Additions: 1, 0·0014 *M* ATP + 0·03 *M* glucose + 0·8 mgm. yeast hexokinase + 0·00005 *M* atractyloside; 2, nil; 3, 0·0014 *M* ATP + 0·03 *M* glucose + 0·8 mgm. yeast hexokinase; 4, 0·0014 *M* ATP + 0·00005 *M* 2,4-dinitrophenol + 0·00005 *M* atractyloside; 5, 0·0014 *M* ATP + 0·00005 *M* 2,4-dinitrophenol. Other conditions as in Table 1

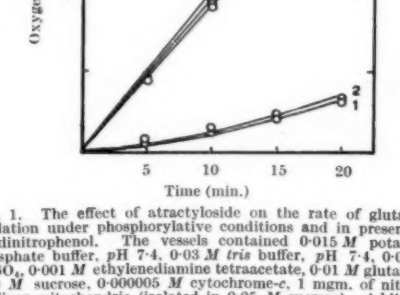

Fig. 2. Inhibition by atractyloside of the oxygen uptake stimulation induced by inorganic phosphate. The vessels contained 0·042 *M tris* buffer, *p*H 7·4, 0·005 *M* $MgSO_4$, 0·001 *M* ethylenediamine tetraacetate, 0·01 *M* glutamate, 0·09 *M* sucrose, 0·000005 *M* cytochrome-*c*, 0·8 mgm. of nitrogen rat-liver mitochondria (isolated in 0·25 *M* sucrose), 0·03 *M* glucose, 0·8 mgm. yeast hexokinase, 0·0014 *M* ATP. Additions at the arrow: 1, nil, or 25 μmoles potassium phosphate + 0·14 μmole atractyloside; 2, 25 μmoles potassium phosphate. Final volume 2·8 ml.; other conditions as in Table 1. The numbers in parentheses refer to the P/O ratios determined for the last 15 min. of the experimental period

Attempts were made to locate the action of the drug on the sequence of phosphorylation reactions. Fig. 1 shows the effect of atractyloside in a ~P acceptors-deficient system activated by 2,4-dinitrophenol. A concentration of this substance, able to produce complete uncoupling of the respiratory chain phosphorylation, completely removes atractyloside inhibition on oxygen uptake with glutamate as substrate; the same is obtained with β-hydroxybutyrate, succinate and malate as substrates.

Atractyloside blocks both uptake of phosphorus and the increase of respiration produced by addition of inorganic phosphate in mitochondria depleted of

endogenous phosphorus by pre-incubation with adenosine triphosphate, glucose and hexokinase[5], with glutamate (Fig. 2) or β-hydroxybutyrate as substrate; consequently, it is likely that systems utilizing inorganic phosphate are particularly concerned with the inhibition of phosphorylation by atractyloside.

Because of its action on phosphorylation reactions, atractyloside appears to be a very suitable drug for work on oxidative phosphorylation; for example, according to Slater[6,7], these results could provide evidence that for the stimulatory effect of 2,4-dinitrophenol in ~P acceptors-deficient systems, inorganic phosphorus is not necessary; indeed, the action of this uncoupling agent is present also with atractyloside, which inhibits phosphorus uptake. The various aspects of the antagonism between 2,4-dinitrophenol and atractyloside are under investigation. This work has been aided by a grant from the Consiglio Nazionale delle Ricerche.

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Comparison between Water and Alcohol Consumption in Six Animal Species in Free-choice Experiments

Great differences exist in the inter-individual fluctuation of the voluntary intake of alcohol by laboratory rats on free choice between 10 per cent alcohol and water[1]. This is also true of laboratory mice[2]. But on the whole the average alcohol consumption within a species of animal is constant. It can be said that each species has its own specific liking for alcohol.

In free-choice experiments the results are usually expressed as the total amount of alcohol consumed. But if the data are intended to give a picture of the preference for alcohol compared with water, it would be clearer to express the results in terms of the alcohol drunk in relation to the total amount of liquid consumed. In this way the preferences of animals of different weight and species can be directly compared.

In this work the consumption of 10 per cent alcohol and water in free-choice experiments in six animal species has been studied. The animal species were: hedgehog (*Erinaceus europeus*), golden hamster (*Cristatus auratus*), guinea pig (*Cavia porcellus*), rabbit (*Lepus cuniculus*), rat (*Mus norvegicus*, albino laboratory stock), and mouse (*Mus musculus*, Swiss albino Webster).

The hedgehogs were given only fish as food, the hamsters coarse oat meal and green forage, the guinea pigs were given hay and turnips, the rabbits hay or fresh grass, the rats a laboratory food mixture and the mice a commercial food mixture. The alcohol and water consumption was noted every day except for the mice, for which it was measured every 3–4 days. The position of the bottles was changed every 2–3 days. The results of the experiments are shown in Table 1.

The hedgehogs preferred water to alcohol solution. Sometimes, however, the animals drank considerable amounts of alcohol, and it could at times be judged from their movements that they were slightly drunk. The hamsters preferred alcohol. They seldom tasted the water and, when they did so, only until they found out that they were drinking from the wrong bottle. The guinea pigs preferred water. The comparatively large consumption of fluid and the wide variations in consumption showed that the animals spilled out some of the fluid; but it could be seen that they visited the water bottle more often and only seldom the alcohol vessel. The rabbits drank about as much alcohol as water. Individual rats differ in their choice between alcohol and water. Most of them prefer water to alcohol, but some prefer alcohol. In some individuals there was a change in preference while the experiments were in progress. What is said about rats is also true of mice.

If an animal cannot taste the difference between alcohol and water or has an equal liking for both, it does not discriminate between them, and the intake of the two drinks is about the same. Rabbits belong to this group of animals. Guinea pigs, rats, mice and hedgehogs discriminate between these solutions and prefer water to alcohol. It cannot be said from these experiments whether the preference for water depends only on an aversion to the taste of alcohol or whether any negative, physical effect of the alcohol is involved. Rats at least have an absolute aversion to alcohol and not merely a relative preference for water, since if alcohol is the only solution available the amount of fluid consumed is lower than when plain water is available[3].

In contrast to the animals mentioned above, hamsters have a pronounced preference for alcohol. This has earlier been shown to be true of deer mice (*Peromyscus c. californicus*)[4] and of the mouse strain Loiseleur and Petit used[5].

In this experiment there is no absolute measure of how much an animal likes or dislikes alcohol. It is only possible to measure how much the animal likes alcohol compared with its liking for water. A third alternative would have altered the results in relation

Table 1. Alcohol and Water Consumption in Six Animal Species in Free Choice Experiment

No. of animals	Animals × days	Species	10 per cent alcohol per day (ml.)	Water per day (ml.)	Percentage drunk as 10 per cent alcohol	Alcohol–water $P <$
12	420	Hedgehog	6·8 ± 0·53	11·9 ± 1·07	36·3	0·0002
10	340	Golden hamster	5·4 ± 0·19	0·7 ± 0·01	88·0	0·0002
8	256	Guinea pig	8·7 ± 0·97	71·2 ± 2·43	10·8	0·0002
8	168	Rabbit	3·1·8 ± 1·32	37·3 ± 1·54	48·3	0·5
12	300	Rat	7·4 ± 0·55	17·5 ± 0·98	29·8	0·0002
10	72	Mouse	10·0 ± 5·08	26·9 ± 5·09	27·1	0·0002

to the preference for the new solution. As Lester and Greenberg[6] have shown, a third bottle containing sugar solution strongly influenced the choice of alcohol. Only if the preference for a substance is strong in every set of alternatives can it be said that the animal has a specific craving for this substance.

The reason for the preference for, or aversion to, alcohol among animals is not known. It was shown earlier that a low original alcohol consumption in a rat can be changed into a strong preference in various ways: by a deficient diet causing metabolic block[7], by damaging liver function[8] or by inducing an experimental conflict situation[9]. In our investigation all these conditions could be excluded. According to one of us[10], the choice of alcohol can be explained by the metabolic type of the animal. Perhaps differences in metabolism can explain the differences in preference for alcohol in the various animal species.

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RADIOBIOLOGY

Urinary Metabolites of ^{14}C-Tetrachloroethylene in Mice

In recent years tetrachloroethylene (perchloroethylene) has rapidly gained in importance as an industrial solvent. Its toxic effects have not been investigated very much, however, and its metabolism is almost unknown. The present work was carried out in order to facilitate the estimation of health hazards caused by exposure to this solvent.

Starting with ^{14}C-acetylene, tetrachloroethylene was synthesized essentially according to the industrial method[1,2]:

$$CH{\equiv}CH + 2Cl_2 \longrightarrow CHCl_2CHCl_2$$

$$CHCl_2CHCl_2 + OH^- \longrightarrow CHCl{=}CCl_2 + H_2O + Cl^-$$

$$CHCl{=}CCl_2 + Cl_2 \longrightarrow CHCl_2CCl_3$$

$$CHCl_2CCl_3 + OH^- \longrightarrow CCl_2{=}CCl_2 + H_2O + Cl^-$$

The crude ^{14}C-tetrachloroethylene was purified by preparative gas chromatography, and the specific activity of the purified product was 0·7 μc./mgm.

Five female mice, one at a time, were exposed for 2 hr. in a sealed flask (2·7 l.) to the solvent vapour in doses of about 1·3 mgm./gm. body-weight. 70 per cent (42–87 per cent) of the solvent was absorbed. In four days about 90 per cent of the absorbed activity was excreted, namely, 70 per cent in the expired air, 20 per cent in the urine and less than 0·5 per cent in the fæces. By chromatographic, autoradiographic and isotope-dilution methods, in part by Jondorf's method[3], the following metabolites were identified in the urine (percentage of total urinary activity): trichloroacetic acid (52 per cent), oxalic acid (11 per cent) and dichloroacetic acid (traces). No monochloroacetic acid, formic acid or trichloroethanol was found. After hydrolysis 18 per cent of the urinary metabolites were still not extractable with ether.

These results indicate the formation of an epoxide further rearranged to trichloroacetyl chloride as one metabolic pathway for tetrachloroethylene:

$$CCl_2 : CCl_2 \rightarrow Cl_2C\!\!<\!\!\overset{O}{}\!\!>\!\!CCl_2 \rightarrow Cl_3C.COCl \rightarrow Cl_3C.COOH$$

This reaction has been previously shown to occur *in vitro* with the oxidation of tetrachloroethylene in the presence of non-biological catalysts[4,5].

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Action of Tetracycline on the Level of Properdin

The influence of antibiotics on the formation of antibodies has been studied by many authors. Recently, Patočka *et al.*[1] published a paper describing the inhibitory effect of tetracycline on the formation of specific antibodies of various types in infections or after immunization with brucellæ, the influenza virus and herpes in laboratory animals. Clinical experience shows that in certain infections caused by pathogenic agents (viruses) tetracycline may have a favourable effect on the course of the disease. We tried to ascertain whether in these cases this is not due to the action of tetracycline on a non-specific defence mechanism.

We therefore investigated the properdin-levels in rabbits daily for one month with intramuscular application of 25 mgm. tetracycline per kgm. body-weight. Determination of properdin was carried out by Isliker and Linder's[2] modification of Pillemer's method. Properdin was determined before the beginning of the experiment and at intervals of 10 days. Before the experiment the average properdin-level was approximately ten units, which is regarded as the normal level. During the first ten days, properdin activity increased to a varying degree in all laboratory animals, on the average to more than double its original value. On the twentieth day we noted a marked fall, in spite of the continued application of tetracycline; on the thirtieth day the level stabilized at about the normal value. Fig. 1 shows the curve of the properdin-level in a group of nine rabbits after statistical evaluation. The experiment was then repeated in a similar manner with six rabbits which were also exposed to X-rays (500 r.). The results obtained in this group were similar, except that the decrease in the properdin-level between the tenth and the twentieth day after irradiation was much more pronounced, frequently below the original level. In the control group of four rabbits the familiar decrease in properdin activity was

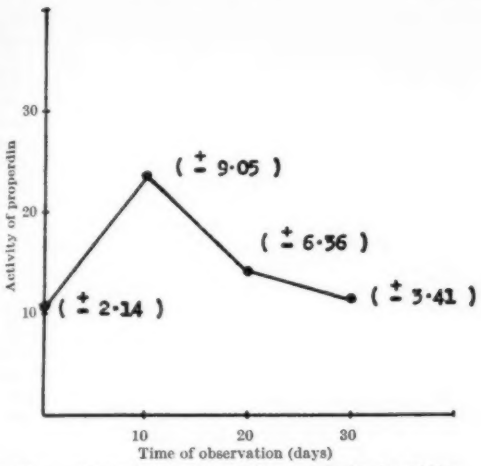

Fig. 1. Effect of tetracycline on the properdin-level in rabbits

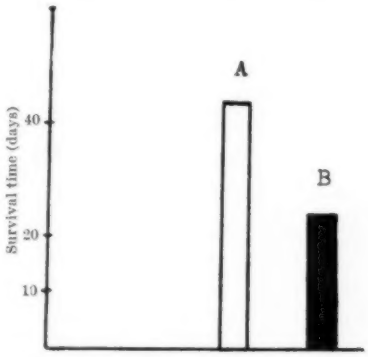

Fig. 2. Effect of tetracycline on the survival-time of irradiated rats. *A*, Group of ten rats treated with tetracycline; *B*, group of ten control rats

observed and normalization was reached on the thirtieth day.

To supplement this experiment, twenty white rats were irradiated (750 r.) and the effect of tetracycline on the survival-time was studied in ten rats against ten control animals. Fig. 2 shows a prolongation of the survival-time through the action of tetracycline. The relation between the prolonged survival-time of irradiated rats under the influence of tetracycline and the activity of the properdin system still remains a matter of conjecture; however, the results could serve as a basis for further work on the prevention of damage due to irradiation and the tetracyclines may be an effective co-factor in the suppression of the damaging effects of ionizing radiation.

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HÆMATOLOGY

Thromboplastic Activity of Red Cells

WHILE a number of workers have reported thromboplastic activity exerted by hæmolysed red cells, intact red cells have been reported to display no such activity[1].

In experiments to be reported elsewhere, it has been found that under conditions more nearly approaching physiological, red cells exert a platelet-like thromboplastic activity of an order approaching that exerted by platelets.

The generation of thromboplastin was investigated first in diluted platelet-rich plasma. It was found that platelets which had thus been subjected to no manipulation exerted a much more sluggish action than that exerted by washed and re-suspended platelets. Twenty minutes might elapse after re-calcification before thromboplastic activity reached its maximum. If the plasma stood at room temperature for 2–3 hr. before re-calcification, this delay was shortened and the maximum activity developed was greater. A clot formed in the incubating plasma, if the ultimate concentration of plasma was more than 1 per cent, and it was found that thromboplastin formation was minimal until this clot formed and was separated. If the clot were allowed to stand for 2–3 min. before it was separated, thromboplastin was still minimal immediately after separation, but then developed rapidly and reached a higher maximum which approached that developed by washed platelet suspensions. Rapidly centrifuged normal plasma or slowly centrifuged plasma from thrombocytopænic patients treated in the same way developed little thromboplastic activity.

Blood was then used instead of plasma. Somewhat greater activity developed in diluted blood than in slowly centrifuged plasma, diluted to give the same plasma concentration. When blood from thrombocytopænic patients was used, diluted blood developed much greater activity than diluted slowly centrifuged plasma. There was little difference in the activities developed by normal blood and by thrombocytopænic blood.

This activity of red cells showed similar characteristics to those of platelets—in that maximum activity was displayed when the blood stood for 2–3 hr. first or when the clot was allowed to stand for 2–3 min. before it was separated; thromboplastin formation was minimal until the clot was separated. The activity was not due to hæmolysis, of which there was no evidence.

Certain discrepancies with previous observations could be resolved.

First, blood from thrombocytopænic patients displays poor prothrombin consumption. This is due to the fact that the red cells exert little thromboplastic activity until they have escaped from the clot. In the prothrombin consumption test centrifugation separates the cells from the serum before much action has been exerted. Little thromboplastic activity is then developed compared with the activity developed when the red cells are dispersed into the serum.

Other discrepancies are that the thrombin generation test of Macfarlane and Biggs[2] shows considerable difference between thrombocytopænic and normal blood, and that washed red cells have been found inactive in the thromboplastin generation test[1].

Investigation of these discrepancies revealed a difference between the behaviour of platelets and of

red cells. While both develop marked activity only after coagulation has occurred, the activity exerted by red cells before coagulation has occurred is much weaker than is that of platelets. When, therefore, platelet activity of other source is minimal and when other conditions are unfavourable to coagulation, the development of red cell activity tends to be blocked. In the thrombin generation test coagulation is retarded, because the blood has not been exposed to surface activation. In the thromboplastin generation, test dilution of the constituents retards coagulation. In both cases when red cells are present, but no platelets, the red cell activity develops only slowly. By varying conditions, however, red-cell activity can easily be demonstrated in both types of test.

The difference in behaviour of platelets and red cells makes a quantitative comparison impossible, unless the conditions are specified. Moreover, since the removal of all platelets tends to block the development of red-cell activity, it might be thought that this activity would be unimportant under physiological conditions. This requires further investigation, but so far the evidence obtained tends to indicate that red cells contribute to coagulation under natural conditions and are partly responsible for the normal coagulation time found in thrombocytopænic conditions. Certain clinical differences between the hæmorrhagic states of thrombocytopænia and of coagulation defects, such as the difference in frequency of hæmarthroses, may be explicable in terms of this red-cell action.

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Thalassæmia in an English and in a German Family

Reports of thalassæmia (major and minor) in British residents of the United Kingdom are few[1-6]. Equally few are reports of the occurrence of this abnormality in subjects of German extraction[7-14].

Recently, we have been studying the frequency of the carrier form of this abnormality (thalassæmia minor) in subjects of Greek, Egyptian, Syrian, Armenian, Italian, Ethiopian and Sudanese (Northern, 'Arab' and Southern, 'Negro') origin, using, as an absolute criterion, the presence of an increased erythrocyte osmotic resistance associated with abnormal morphological characteristics of the erythrocytes[15,16]. During our testing of healthy school children in Khartoum for this purpose we detected one English and one German girl whose erythrocytes repeatedly showed an increased osmotic resistance and, on unstained peripheral smears, anisopoikilocytosis and microcytes. Both girls (aged nine years and thirteen years respectively) were in good general health and appeared normal on clinical grounds. The abnormality was not found in fifteen other British and five other German children tested.

Similar studies carried out on the parents of the English girl (their only child) revealed that both carried the same abnormality and that both were in good general health. The mother was born in Cornwall and was of mixed Spanish–Irish–Cornish stock; she has a brother and a sister both of whom are in good health. The father was born in Malta of British parents. There is a possibility that there was some French admixture in his ancestry.

Examination of both parents of the German girl and of one brother showed that the father was carrying the abnormality and that the mother and brother were normal. The mother was born in Kothen, Anhalt, had a fair complexion and looked and felt well. She was of 'pure' German stock. The father was born in Korrig, Saarburg, and was of a dark complexion with heavy bushy eyebrows. He had suffered from malaria and had occasionally complained of easy fatigability. A recent hæmoglobin test yielded results within normal limits. His sister and his mother are said to have a similar dark complexion. His family had had close ties in Lorraine some two centuries ago.

The evidence of ties with continental Europe in the ancestry of the three parents having the thalassæmia abnormality in these two families raises the possibility that the abnormality entered both these families through their foreign antecedents. It is difficult to prove this point in the absence of any systematic work on the frequency of this abnormality in Spain and France as well as in Great Britain and Germany. Chernoff[17] lists only five references in the literature to thalassæmia in Spanish subjects, while there are only two reports of this abnormality in French subjects[18,19].

It is to be hoped that the significance of the thalassæmia abnormality in these countries will emerge in the not too distant future.

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GERONTOLOGY

Expected Rate of Senescence and Age-dependent Mortality in Fish

It is usual in fisheries investigations to assume that the mortality of wild fish from natural causes does not rise significantly with age, and calculations on this basis give reliable results for most economically important species[1-3]. Age is sometimes invoked to explain the disappearance of fish[4]: at high ages a number of species show a decline in numbers which

might be due to natural ageing[5,6]; but for long-lived species the inference is that few individuals, especially in fished populations, live long enough for the effect of age to be important.

The rate of ageing has been measured actuarially in the guppy (*Lebistes*) on a large tank-bred population[7–9]. In females of this species, growth is 'indeterminate' as in larger fish, but the survival curves under conditions of medium growth are very like those of small mammals, with a limiting age of about 2,000 days. It is of interest to consider: (1) whether the time-relations of growth and senescence can be the same in a small as in a large species; (2) whether the eventual rate of senescence in long-lived fish can be inferred from the figures for *Lebistes*.

Taking the data of *Lebistes* it is possible to construct curves for other species, showing what would happen to ability to survive if hypothesis (1) held good. Fig. 1 gives the growth-curves of 87 tank-bred female *Lebistes* by batches from an experiment still in progress, with the observed survival-curve for the whole sample to 1,100 days; the rest of the curve is supplied from previous series under similar conditions, up to 2,000 days. The median survival is about 1,140 days.

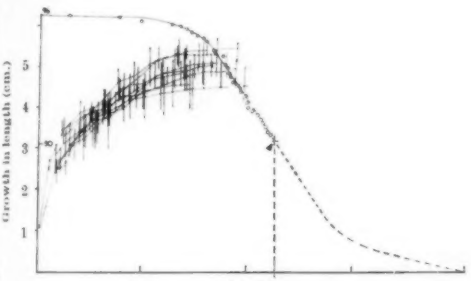

Fig. 1. *Lebistes* females

In Fig. 2, the growth-curves of 4 species in the wild (*Gasterosteus*[10]; cod, plaice[1]; hake[11]) are plotted on time-scales which bring their growth in length within the limits of the curve for *Lebistes*. The conversion factors for time are as follows:

500 days (*Lebistes*) = 1·37 years			
Gasterosteus	1·25 years	×	0·91
Cod	5·0		3·65
Hake	10·0		7·3
Plaice	15·0		10·95

If these relationships hold good, populations with these growth-curves would be expected to have declined by a quarter, from senescence alone, by 2·5, 10, 20 and 30 years respectively, and the four species would have limiting ages of 5, 20, 40 and 60 years respectively. *Gasterosteus* in the wild does in fact live 2–3 years, with a few individuals surviving longer[10]. In the other species, if senescence were related to growth as in the guppy, senile mortality would not be significant within the normal expectation of life in fished populations—the modal age of plaice at catching being about 5 years[1]. The limiting ages seem likewise reasonable, except that on a basis of size one might expect the extreme life of cod to be more than 20 years. These approximations make no allowance for slower growth, however. The guppy population used in this experiment had a rather high rate of growth, and mortality at lower rates increases much more slowly. Since the relation between growth-rate and mortality increase may not be strictly linear, and since no wild population is likely to be as well fed as tank-reared fish in a laboratory, the actual survival-times in the wild may in fact be higher, but are unlikely to be less, than the approximation suggests.

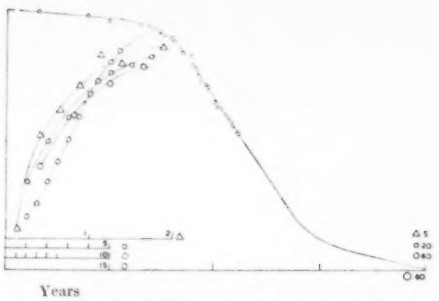

Fig. 2. △, Stickleback; □, cod; ○, hake; ◇ plaice

There seems accordingly to be no reason from existing data to postulate a different pattern of mortality for small as against large fish. The longest-lived freshwater fish appear to be the sturgeons, and for these the same transformation gives a limiting age of about 120 years, which agrees with the trend of recorded ages[12,13].

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ALEX COMFORT

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Frequency of the 'Alpha' Rhythm, Reaction Time and Age

THE preliminary investigation described in this communication was suggested by the observations that (*a*) older people respond to a stimulus more slowly (simple reaction time is longer than in young people)[1–4], and (*b*) the dominant rhythm of the electroencephalogram, the 'alpha' rhythm, is slower in older people than in young[5–7]. These findings raised the question of whether 'alpha' frequency might be a factor responsible for differences in reaction time. The present experiment was designed to discover if simple reaction time is related to 'alpha' frequency of the electroencephalogram recorded during the time which elapses between a stimulus and a response.

Electroencephalograms were recorded from 18 subjects resting in a semi-reclined position, with eyes closed, in a darkened room. The subjects were asked to remain as alert as possible and told that, from time

to time, they would hear a sound (250 cycle tone, well above threshold) to which they should respond as rapidly as possible by uttering a sound ('pow'). No forewarning was furnished to announce the presentation of this stimulus. A signal corresponding to the time the sound appeared was recorded on the chart of an Offner type *D* electroencephalogram machine along with the electroencephalogram tracings and a signal from a microphone which coincided with the subject's response. Bipolar electroencephalograms were recorded from left and right hemispheres. Each pair of surface electrodes was attached to the scalp so that one electrode was over the occipital and the other over the frontal area of the same hemisphere, with each electrode approximately $1\frac{3}{4}$ in. from the midline. An electrode on the arm served as a reference.

Thirteen of the subjects studied (18–72 years) yielded sufficiently regular and artefact-free 'alpha' rhythms to make measurement of frequency possible. The first peak or trough of a wave recorded immediately after the stimulus, and the last peak or trough recorded before the response were located, and the distance between them was measured and converted into time. Dividing this value by the number of cycles described yielded a number equal to the mean period of the 'alpha' rhythm. Only clearly defined waves were considered; cases where uncertainties arose regarding identification of peak or trough or number of cycles were excluded. In general, the mean values from left and right hemispheres did not differ. Whenever differences occurred the shortest 'alpha' period was arbitrarily used in the analysis. Reaction times were measured from the oscillograph chart and converted into time.

A total of 294 observations was obtained from the 13 subjects. The Pearson product-moment correlation (r) between reaction time and 'alpha' period was 0·38 ($P < 0·001$). Of these observations, 78 were obtained under conditions where each subject was requested to exert maximum effort in staying alert and responding rapidly. In this case, the reaction times were shorter and showed a decrease (not statistically significant) in variability. These data are plotted in Fig. 1; in this instance $r = 0·55$ ($P < 0·001$).

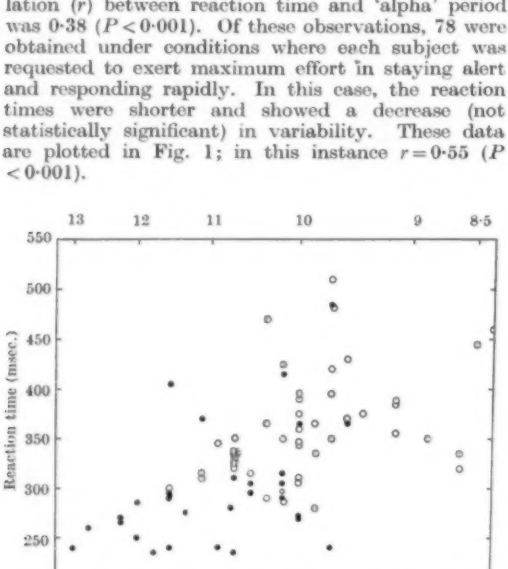

Fig. 1. Reaction time plotted against 'alpha' period. Numbers at the top of the graph refer to the corresponding 'alpha'-rhythm frequencies in c.p.s. Solid circles signify observations from subjects 18–49 years; open circles, subjects 53–72 years

Fig. 1, of course, is purely descriptive and does not distinguish between variances of individual subjects and the variance of the group. These data therefore were analysed by computing each subject's mean reaction time and mean 'alpha' period and correlating these measures. The rank-difference correlation was 0·81 ($P < 0·01$). Finally, the subjects were split into two arbitrary groups, with 8 subjects, 18–49 years of age, in one, and 5 subjects, 53–72 years of age, in the other. Mean reaction time for the younger group was 295 msec., for the older group 367 msec. ; the difference of 72 msec. was significant ($P = 0·05$). The mean 'alpha' period for the younger group was 90·9 msec., for the older group 102·7 msec., and the corresponding difference was also significant ($P < 0·02$).

Thus, although data from only a small number of subjects were available, this investigation reveals the presence of a significant relationship between a person's average 'alpha' period and average reaction time: the longer the 'alpha' period (slower 'alpha' rhythm), the slower the response. It also suggests that older subjects with longer reaction times have correspondingly lower 'alpha' frequencies in the interval between stimulus and response than younger subjects with shorter reaction times. While no causal relationship is implied by the correlations, the results nevertheless lead to the speculation that the 'alpha' cycle, or some multiple of it, may serve as the unit of time in the programming of the nervous system. This possible implication is sufficient to warrant verification of our findings and further study. We need to know, for example, what specific part is played by age in this relationship, and whether the relationship holds in individual subjects.

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BIOLOGY

Effect of Gibberellic Acid on Growth and Indoleacetic Acid Oxidase Activity of *Lupinus albus* L. exposed to Different Photoperiods

The leaves of 14-day-old *Lupinus albus* plants (variety *Gyulatanyai édes*) exhibit a high indoleacetic acid oxidase activity if grown under short-day conditions. In the leaves of plants exposed to long days and exhibiting more intense growth indoleacetic acid oxidase activity can be detected only after dialysis of the extracts[1]. The total phenol content of short-day exemplars is lower than that of long-day ones[2] (Table 1). Therefore, it is very probable that the indoleacetic acid oxidase activity of *Lupinus* seedlings is influenced by the phenol content of the leaves, and indirectly the growth response of plants to different photoperiods depends on the level of phenolics.

Table 1. Effect of Treatment with Gibberellic Acid on the Growth of 14-day-old *Lupinus albus* Seedlings and on the Indoleacetic Acid Oxidase Activity and Phenol Content of their Leaves under Different Photoperiods

Photo-period	Treatment with gibberellic acid	Total heights of the overground parts (cm.)	Total phenols (expressed as 10^{-1} μgm. ferulic acid in mgm. dry matter)	Indoleacetic acid oxidase activity (μl. O_2/hr./ mgm. dry matter)
Short day	−	61·6	2·57*	9·5
	+	90·4	2·40	9·3
Long day	−	107·1	3·23*	0
	+	136·8	3·28	0

* The difference between the phenol content of treated and control plants is significant only between short-day and long-day plants.

It has been shown that gibberellic acid *in vivo* reduces the activity of indoleacetic acid oxidase[3,4] probably by enhancing the synthesis of phenolic enzyme inhibitors[5,6]. Furthermore, according to Pilet, gibberellic acid exerts a direct inhibitory effect on indoleacetic acid oxidase and thereby creates a higher auxin level *in vivo*[3,7]. Therefore, a study of the effect of gibberellic acid on the indoleacetic acid oxidase activity, content of total phenolics and plant growth of 14-day-old *Lupinus albus* seedlings seemed to be warranted.

Seeds of this plant were germinated for 4 days at 25° C. in Petri dishes on filter paper moistened with gibberellic acid solution (10 mgm. in 1,000 ml.). The seedlings were then grown in pots and exposed to short-day (8 hr. daylight) or long-day conditions (8 hr. daylight + 16 hr. supplementary illumination by a 100-W. filament bulb; 2,000 lux) respectively. After the expansion of the cotyledons, the growing point of each plant was treated with gibberellic acid (2 μgm.). Controls were germinated on distilled water and the seedlings were similarly treated with distilled water. At the age of 14 days, the content of total phenolics[8] and the activity of indoleacetic acid oxidase[9] in the first leaf pairs were determined. The height of the plants was also measured. The results are summarized in Table 1.

Results similar to those shown in Table 1 were obtained with plants grown in nutrient solutions supplemented with 10 mgm. gibberellic acid/1,000 ml.

It may be seen that the growth of seedlings is promoted by gibberellic acid both under short- and long-day conditions; however, the stimulation is more pronounced with plants exposed to short days[10] and the effect is apparently additive[4].

The amount of total phenolics and the activity of indoleacetic acid oxidase is not altered by gibberellic acid treatment. The same results were obtained with gibberellic acid concentrations as high as 100 mgm./ 1,000 ml. The results clearly indicate that the stimulated growth of *Lupinus* seedlings evoked by gibberellic acid cannot be due to the direct or indirect (via the stimulation of phenolic inhibitors) inhibition of indoleacetic acid oxidase. It seems more likely that the effect is connected with the elevation of the endogenous auxin-level or with the neutralization of growth inhibitors[11–13]. Isotopic work of Fang *et al.*[14] also supports this idea.

The above results obtained with *Lupinus albus* might seem strange in the light of the available published data. However, we refer to observations by Hillman and Galston indicating that the indoleacetic acid oxidase of *Lupinus albus*, in contrast to that of the pea plant, cannot be influenced by red–infra-red irradiation[15].

Watanabe and Stutz[4] were also unable to show the effect of gibberellic acid on the indoleacetic acid oxidase of *Lupinus albus* leaves. However, they used 70-day-old plants and indoleacetic acid oxidase activity could not be detected in the leaves of control plants either. According to our results, this might be due to the high level of inhibitor(s)[8].

Thanks are due to Gy. Németh for the plant material and to G. L. Farkas for a sample of ferulic and gibberellic acid.

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Relative Volumes of Cytoplasm and Vacuole in *Nitella*

In order to understand the mechanism by which various organic molecules are accumulated by the vacuoles of the green alga *Nitella*, it was necessary to determine accurately the relative volumes of cytoplasm and vacuole in the cell. The unusually large size of these cells has made the genus popular as a tool for the study of biological transport mechanisms[1–5]. Single cells can be obtained up to nearly 2 mm. in diameter and more than 10 cm. in length. The contents of the large vacuole can be obtained relatively free of cytoplasm by cutting off one end and gently squeezing the solution on to a microscope slide or into a capillary para tube. There seems to be little agreement in the literature concerning the relative volumes of cytoplasm and vacuole. Thus Brooks[1] arrived at a value based on intracellular distribution of radioactive potassium and estimated 33 per cent cytoplasm, 67 per cent vacuole. Holm-Jensen *et al.*[2] measured the depth of the immobile chloroplast layer plus the streaming cytoplasm by differential focusing with a water-immersion objective, and the diameter of the cell with a micrometer eyepiece in a dissecting microscope. Calculations from their results give values of 10 per cent cytoplasm, 90 per cent vacuole. Recently, MacRobbie and Dainty[4], measuring the rate of loss of sodium-22 from *Nitellopsis* cells previously loaded with the isotope, calculated that the cytoplasmic compartment consisted of about 3 per cent of the cell. In a similar fashion, using potassium-42, Diamond and Solomon[5] arrived at values of 1·8 per cent cytoplasm.

For our experiments we used *Nitella opaca* Ag. gathered from a stream in southern Sweden and maintained in tap water. We used several direct

methods to measure the volumes of the two compartments. In preliminary experiments we cut the end of a cell and squeezed out the total contents directly into a capillary tube. The tube was sealed and centrifuged. Volumes of the fractions were measured by projecting the image of the tube and contents into tracing paper, cutting out the paper, and weighing the areas. The total cytoplasm constituted 8·7 per cent of the recovered volume. Three distinct fractions were observed. The average volumes for the eight cells measured were: dense green chloroplast fraction, 3·8 per cent; translucent cytoplasm (probably the streaming layer), 4·9 per cent; clear supernatant vacuole, 91·3 per cent. There were two important sources of error in this technique which tend to give low values for the cytoplasm. It was impossible to get all the chloroplast fraction out of the cell, and some of the green material adhered to the walls of the capillary tubes.

In order to overcome these difficulties we placed intact cells in glass tubes, sealed one end, filled with water, and centrifuged at high speed with the tubes in a horizontal position. The three cellular fractions separated very distinctly. By projecting the image of the centrifuged cell into tracing paper, as above, we measured the following average values (per cent) for eight cells: chloroplast fraction, 4·1; streaming cytoplasm, 5·8; total cytoplasm, 9·9 (range, 8·8–11·8).

Since the technique of centrifugation probably compresses the *in situ* volume of the chloroplast and streaming cytoplasm fraction, we measured the dimensions of the sum of both the chloroplast and cytoplasmic layer with a calibrated focusing micrometer on a microscope with a water-immersion lens. Then, by measuring the diameter of the cell with a micrometer eyepiece, it is possible, assuming the solid geometry of right cylinders, to calculate the volume occupied by the two fractions. For eight cells the values are: range of diameter, 375–550μ; range of depth of cytoplasm, 11·2–16·1μ; average volume of cytoplasm, 12·2 per cent (range, 9·3–14·1 per cent).

In the fourth technique, we used the fixation of cells by various methods among which the Navaschin–Karpechenko technique appeared to cause the lowest degree of distortion, though invariably leading to some plasmolysis. Measurements were obtained from photomicrographs of microtome sections and suitable calculations made. The dimensions of a typical cell were found to be: thickness of cell wall, 7·9μ (sections of fresh material gave somewhat higher values); thickness of total plasmatic layer, 17·8μ; overall diameter of whole cell, 460μ. The relative volume of cytoplasm is therefore 14·4 per cent.

The last three methods used give values for cytoplasmic volume of 9·9–14·4 per cent, and are in rather close agreement. Both in living material and in the microtome sections the innermost part of the cytoplasm was seen to have a somewhat foamy appearance, which explains, at least in part, the difference between the values obtained by centrifuging and those obtained by direct measurements. We can say, with some degree of certainty, that the cytoplasm occupies at least 10 per cent of the physical volume of the cell, as had been suggested by Holm-Jensen *et al.*[2]. The value is at great variance with that of MacRobbie and Dainty[4] and Diamond and Solomon[5]. There is no doubt that there is some 'compartment' comprising 2–3 per cent of the cell which loses alkali metal ions in the fashion described by these workers. This compartment is not, however, the total cytoplasm. Rather, it might be the matrix of the immobile chloroplast fraction. As judged by qualitative microscopic observation, we estimate that the matrix occupies approximately 2–3 per cent of the volume of the cell.

Our work suggests that some compartments of the cell are not limited by the finite membranes of the tonoplast and plasmalemma, but should be considered as volumes of the cell which differ in physical and chemical properties which influence their permeability behaviour.

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Inoculation of Pine Stumps against Infection by *Fomes annosus*

Recent trials have shown that inoculation of pine stumps with oidia of *Peniophora gigantea* prevents infection by spores of the fungus *Fomes annosus*, causing root-disease of conifers. *P. gigantea* is an active wood-rotting fungus which under natural conditions often competes with *F. annosus*, but for various reasons does not provide consistent control. It is a far more effective competitor than wood-inhabiting microfungi such as *Trichoderma viride* and *Penicillium* spp., which seldom colonize a fresh stump surface at all freely.

To prepare cultures of *P. gigantea*, pine wood-blocks of 3 cm. × 3 cm. × 1 cm. are autoclaved in batches of one hundred, inoculated with a suspension of oidia and incubated at 22° C. for 10 days. They are then sealed individually in small, sterile polythene bags, in which condition they remain usable for many months. The suspension of oidia is prepared by shaking a wood-block culture with about 100 ml. tap water for 1 min. It is then diluted 5–10 times and sprayed on to the stump surface by means of a large syringe directly after felling. According to the size of stump, 3–5 strokes suffice: these deliver 2–3 ml. suspension containing some 1×10^6 viable oidia. It is desirable to use a fresh suspension each day. In cold weather oidia may be suspended in 10 per cent ammonium sulphate, which considerably depresses the freezing point without greatly affecting spore viability.

In ten experiments, involving some 400 stumps of *Pinus sylvestris* or *P. nigra* var. *calabrica*, samples taken after 6–12 months showed that inoculation had consistently prevented infection by *F. annosus*, whether derived from natural sources or from adding up to 3,000 spores per stump. About 90 per cent of inoculated stumps produced sporophores of *P. gigantea* within a year, thus increasing local spore production of this useful competitor. The treatment was also effective in preventing growth of *F. annosus* into

stumps, the roots of which were inoculated with the parasite soon after felling: in five stumps excavated a year later, no roots were found to contain *F. annosus*. By contrast, in root-inoculated stumps treated with creosote or urea at the time of felling, two-thirds and one-third of the roots, respectively, were occupied by the parasite. With naturally infected pine stumps, however, *P. gigantea* was rather less effective because it seldom replaced *F. annosus* in resinous roots already dead at the time of felling.

This method should prove useful in preventing entry of *F. annosus* into first-rotation pine stands of special value, such as experimental plots; it might also find wider application in young forests where *P. gigantea* is as yet uncommon. Despite the limitation mentioned, inoculation of stumps with *P. gigantea* may well be preferable to treatment with chemicals[1] in stands where *F. annosus* has already become established. Preliminary trials suggest that it may be possible, by selecting appropriate saprophytes, to protect stumps of other conifers against infection by *F. annosus*.

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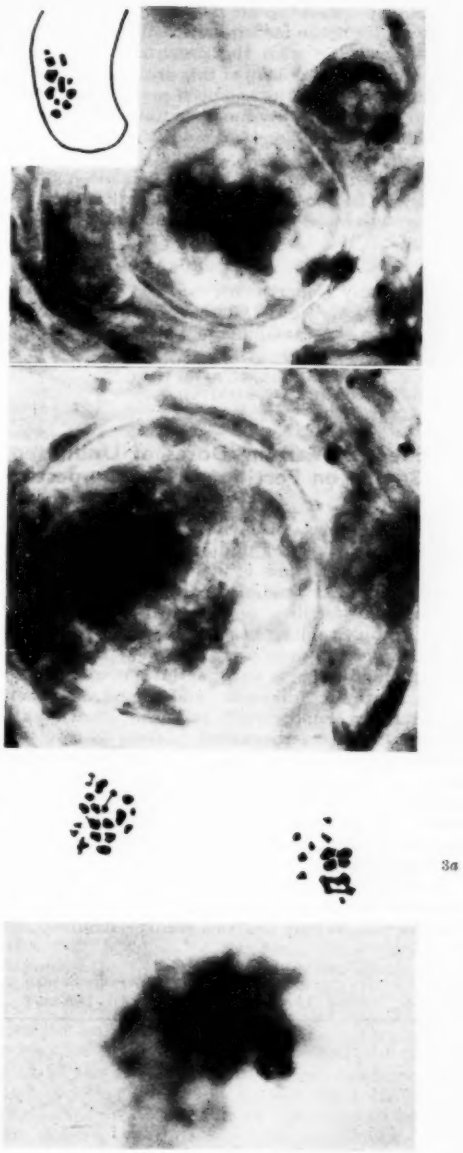
Meiosis in the Oogonium and Antheridium of *Pythium debaryanum* Hesse

Aceto-orcein squash preparations of a culture of *Pythium debaryanum* Hesse were found to have nuclei in division in the oogonia and the antheridia (Figs. 1 and 2). The stage of division was usually rather more advanced in the oogonium than in the antheridium. The oogonia had 2–6 nuclei undergoing division, the antheridia usually one, occasionally two.

The haploid number of chromosomes is approximately twenty (Figs. 2, 2*a*), but an exact count has not been possible so far, partly because of the smallness of the chromosomes and partly because of certain irregularities. At first it was assumed that these divisions were mitotic, but a closer examination disclosed side views of configurations at early anaphase with loops such as would be expected to result from interstitial chiasmata. Also, certain irregularities such as early separation of failure of pairing indicated that these divisions were meiotic. No metaphases were seen in the hyphæ or sporangia, even after camphor treatment, and it is concluded that vegetative nuclei do not normally pass through an ordinary metaphase stage when dividing. The occurrence of metaphases in the oogonia and antheridia therefore indicates that these may be special divisions, that is, meiotic divisions.

Full confirmation of this was obtained when configurations of more than two chromosomes were obtained (Figs. 3, 3*a*). Such configurations could only occur in meiosis. This indicates that *Pythium debaryanum* is a structural hybrid, or a polyploid which may well account for the failure of previous attempts to germinate the oospores.

The Peronosporales have generally been considered to be haploid with meiosis occurring in the first division of the oospore nucleus. However, there is little or no direct evidence for this view. It is very unlikely that *Pythium debaryanum* should have a different life-history from the rest of the group,



Figs. 1–3. 1. Oogonium and antheridium, metaphase I in antheridium; (1*a*) drawing of metaphase. 2. Oogonium and antheridium. Oogonium with 4 metaphases, 1 in focus. (2*a*) Drawing of metaphase plate. 3. Side view of metaphase I in oogonium showing a multiple configuration. (3*a*) Drawing of metaphase. (Figs. 1 and 2 × *c.* 2,600 ; Fig. 3 × *c.* 4,300)

and I feel sure that further work will show that the Peronosporales and probably the Biflagellatae as a whole are diploids in the vegetative stage with meiosis occurring in the oogonium and antheridium. It is very suggestive that two successive divisions have been reported to occur in the oogonium and antheridium of *Albugo* sp.[1]. The genetical work on the Biflagellatae is very scant, but according to Raper[2] the complex pattern of sexuality in *Dictyuchus*

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reported by Couch[3] could be accounted for by polyploidy or duplicate factors. This suggestion of Raper is in accordance with the present observations, in indicating that members of this group may be diploid rather than haploid as has been previously supposed.

I am indebted to my husband and Dr. B. J. Harris for helpful discussions and to the latter for the photomicrographs.

Note added in proof. Meiosis has been observed recently in the oogonium and antheridium in *Phytophthora cactorum*, which has approximately nine bivalents.

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Effect of Varying Doses of Undiluted Semen on Fertility in the Domestic Fowl

Several reports[1–3] have appeared on the relationship between dose of undiluted semen and fertility in chickens, yet there is little evidence on the minimum volume of undiluted semen necessary (*a*) to produce any fertility, and (*b*) to establish the maximum or optimal level of fertility. The relationship between spermatozoa number and fertility has been investigated by using varying doses of semen diluted with synthetic diluents and seminal plasma[4,5]. However, recent work[6,7] indicates that synthetic diluents and even seminal plasma can alter the metabolic rate and perhaps even the life-span *in vivo* of fowl spermatozoa. This is a brief report of an experiment undertaken to establish the minimum volume of undiluted fowl semen required to produce any fertile eggs, and minimum volume necessary for optimal fertility.

Semen was obtained from 15 males selected randomly from each of two random-bred strains—the Ottawa S.C. White Leghorn control strain[9] (Leghorn control) and the Ottawa meat control strain[10] (meat control). All inseminations were made into random samples of females from two selected strains of S.C. White Leghorns[9] that were known to be similar in production traits. Both strains of females were highly fertile.

Semen was collected from each male and pooled by strain after rejecting samples which were contaminated and contained 'clear watery fluid'[7]. Fifteen doses of semen were used (Fig. 1) varying from 0·0002 ml. to 0·15 ml. The experiment was repeated for six days. Each day two hens were inseminated with each dose from each of the two strains of males. Twenty-four hens were inseminated with each dose for a total of 360 inseminations. Only hens that had laid already on that day were inseminated.

Glass pipettes fitted to a 1 ml. tuberculin syringe by rubber tubing were used to measure and deliver the semen. Four different-sized pipettes were used, the smallest graduated to 0·0002 ml. with a diameter of 0·25 mm. It was recognized that the error in measurement and delivery of the smallest dose was quite high, but not greater than ± 0·00005 ml.

Following the single insemination, all hens were trap-nested and the eggs were marked, dated and stored in a holding room at a temperature of 50° F. and with a relative humidity of 85–90 per cent. They were incubated weekly, until all hens were completely infertile.

Differences between the two strains in semen density were not significant. This confirmed our previous findings[10]. Therefore, the data on spermatozoa number from the two strains were pooled. Fertility was measured as the duration of fertility in days, following a single insemination[8]. The results are plotted in Fig. 1, showing both the size of the dose and the number of spermatozoa per dose.

The differences between the strains were highly significant. Differences between strains and between breeds in duration of fertility are a relatively widely spread phenomenon[2,11,12].

The duration of fertility for the Leghorn control and for the meat control increased with an increase in the semen dosage up to 0·01 ml. and 0·006 ml. respectively. Although there was a slight increase in fertility with doses above these levels, it was not significant. With doses as small as 0·0002 ml. some fertility resulted in both strains.

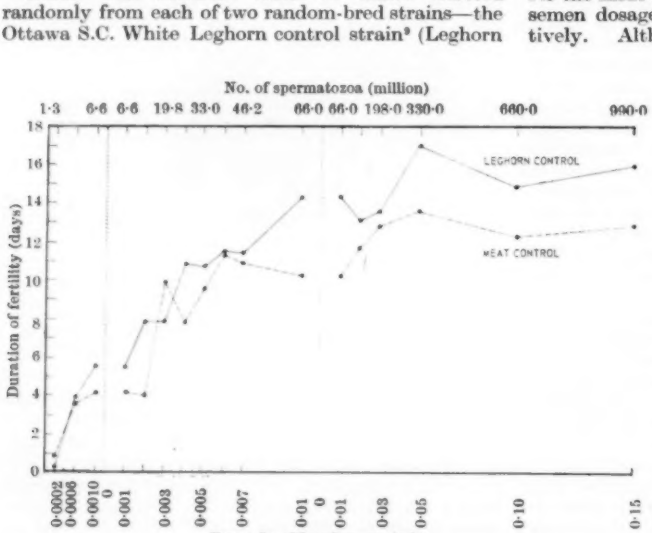

Fig. 1. Relationship between the dose of undiluted semen (number of spermatozoa) and the duration of fertility in two strains of domestic fowl

The relationship between fertility and spermatozoa number should be considered in the light of recent developments in the technique of semen collection[8,13]. Fowl semen collected free of contaminating fluids contains, on the average, six to seven million spermatozoa per ml. of semen[8,10]. This is double the concentration reported by most other workers who, no doubt, were using semen contaminated to some degree[4,5]. In this present experiment the minimum number of spermatozoa required for optimal fertility was 40 and 66 million for meat control and Leghorn control, respectively. These estimates are much lower than those reported by others[4,5]. There is little doubt that the use of careful semen collection and insemina-

tion techniques have contributed to the results obtained.

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Copepod *Ommatokoita elongata* (Grant) in the Eyes of the Greenland Shark—a Possible Cause of Mutual Dependence

In July 1959[1], and in July–August 1960, I had the opportunity to take part in Greenland shark fishery with Norwegian commercial sealing vessels in east Greenland waters. Data on this shark were collected, supplemented with information received from experienced Greenland shark fishermen.

The sluggishness of this shark is proverbial, and it is to many a puzzle how such a sluggish fish is able to capture live prey.

The information received from the fishermen tends to show that, besides many other marine animals, the char, *Salmo alpinus*, has been repeatedly found in stomach contents of this shark, even so freshly swallowed that it could be used for human consumption on board. According to information received from several skippers, some of the chars found in shark stomachs had been cut by the shark's teeth when captured, and in such cases their tail parts were invariably missing in the stomach contents. This naturally leads to the assumption that the char has been swallowed head foremost by the shark. It is believed very strongly by Norwegian fishermen, who have been fishing for Greenland sharks, that its potential prey is lured to swim toward it by the 'white things', that is, the copepod *Ommatokoita elongata*, attached to its eyes. The copepods are said to be luminous by some fishermen.

The infestation with this copepod in south-east Greenland waters was found to be as follows:

Of the 1,505 sharks inspected, 1,270 (84·4 per cent) were infested in both eyes, 218 (14·5 per cent) were infested in one eye only, and only 17 sharks (1·1 per cent) were not infested. It is, however, possible that copepods have been lost during the haul or have been too small to be observed easily.

One copepod in each eye is the rule, but in some cases, included in the numbers given above, two, or rarely more, specimens were attached to one eye. Very rarely specimens were found attached to the skin near the eyes. The copepods are firmly attached to the cornea by a small disk. Numbers of small circular marks on the cornea are thought to be scars left by previous infections. The copepods were found to vary in length from 3–4 mm. to about 70 mm., including the egg sacks. The smallest copepods had evidently become attached recently, and there is thus reason to believe that uninfested sharks were only temporarily free of this copepod. The copepods are whitish-yellow in colour, and in particular adult specimens with large egg sacks are very conspicuous. The cornea was often found to be opaque, and in cases of heavy opacity, copepods were not found to be present.

Table 1

1959	Total	*O. elongata* Both eyes	Right eye	Left eye	Absent	Not examined
Umivik Fjord	264	205	21	21	2	15
Umivik Bank	10	3	2		1	4
1960						
Umivik Fjord	664	519	49	50	9	37
Umivik Bank	244	173	11	12	2	46
Bank area east of Kangerdlugsuak Deep	2	2				
Off Deception Island	473	368	23	29	3	50
	1,657	1,270	106	112	17	152

According to the data collected, this copepod appears to be equally common in both small and large sharks. Differences in infestation relative to sex were very small. As is apparent from the list above, only small variations in infestation are observed between the different fishing localities.

It may be added that mainly small and medium sharks of both sexes were caught in the fjords and near land, while only large fish, from about 3·5 m. to nearly 5 m. in length, were caught on the banks. These were, with one exception, females only.

I have not been able to confirm the fishermen's belief that the copepods are luminous. No bioluminescence could be observed by the human eye in specimens kept in sea water in total darkness.

O. elongata is grouped in the family Lernaeopodidae which, according to Harvey[2], is not included in any of the two copepod sub-orders, Calanoidea and Cyclopoidea, to which bioluminescence seems to be restricted. It is therefore unlikely that *O. elongata* is able to produce light. However, histological examination has not been undertaken.

It may be argued that when the amount of light present in the sea is above a certain minimum, the whitish copepods will show up against a dark background. However, the effect of the tapetum lucidum of the shark's eyes in weak light conditions is not known.

It is generally accepted that many fishes, such as the angler fish (*Lophius piscatorius*) and ceratioid deep-sea fishes, use the first dorsal fin ray as a lure or 'fishing rod' for attracting prey[3]. Although the fishermen's assumption that the copepods in the eyes of the Greenland shark serve a similar purpose sounds rather far-fetched, it should not be entirely excluded. But if such should really be the case, of which I am so far not convinced, then the copepod is of some benefit to its host, and a mutual dependence, which may be classed as a case of mutualism, may be anticipated to exist between the two.

I am fully aware that this view is highly speculative, but it is felt to be of sufficient interest to attract biological attention, in order that other workers who

have the opportunity to add further knowledge may do so.

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Occurrence of *Eutrichocheles modestus* (Herbst) in the Near-shore Waters of Porto Novo, South India

Faunal studies of this region on the Coromandel Coast have been in progress for some years. Some interesting finds have been reported[1–3] recently, after the establishment of a marine biological laboratory at Porto Novo (11° 29′ N. and 79° 47′ E.), and this communication relates to the occurrence of the extremely rare decapod crustacean *Eutrichocheles modestus* (Herbst) (Fig. 1). A specimen 80 mm. in length (from the tip of the rostrum to the posterior extremity of the telson) was first obtained from a seine net operated by local fishermen at about the 10-fathom line (about 4–5 miles from shore) in February 1960. This area is well within the limits of the continental shelf, which extends to about 20 miles along this coast. Another specimen measuring 75·2 mm. in length was obtained in January 1961.

From a survey of the literature it would appear that only two specimens of this decapod have so far been obtained, and the exact source of neither of these is known with certainty. The first specimen described by Herbst (1794)[4] as *Cancer modestus* was probably taken in the East Indies, and the other specimen on which Wood-Mason (1875)[5] based the present generic name was caught somewhere off the Arakan coast of Burma. The latter specimen seems to be the only example in existence now[6]. With the present discovery of *Eutrichocheles modestus*, de Man's statement[7] "that this extremely rare form will probably prove to occur also in the Indian Archipelago" would appear prophetic.

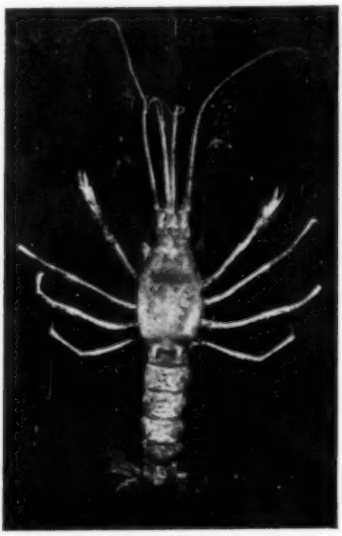

Fig. 1 *Eutrichocheles modestus* (Herbst)

The main features of the specimens from Porto Novo conform very closely to the earlier descriptions, but the following supplementary observations may be made. The fresh specimens appeared rather pink in colour and with preservation they have tended to become white. One is therefore reminded of the red pigment mentioned by Herbst[4] and the white colour referred to by Chopra[6]. Further, the cornea appears to be pigmented unlike what Chopra observed. Again, in our specimens the rostrum ends in a rather sharply pointed tip, and with regard to the abdominal segments, the teeth at the anterior margin of the fifth are rather indistinct compared with those on the third and fourth segments. There is a median groove in the telson running from the notch at its posterior margin, to the middle, where runs a transverse row of spines. The exopod of the uropod also has hairs. This feature was described by Chopra[6].

Unlike the Indian Museum specimen, the right cheliped and not the left is the bigger in our material and both the chelipeds are profusely covered with hair-like outgrowths. The nature and distribution of the tubercles and spines in the different regions of the chelipeds would also appear to vary from what has been described by the earlier authors.

When more specimens of this curious creature become available a detailed investigation of its anatomy will be undertaken, when it may also be possible to discuss its systematics in a more precise manner than has hitherto been possible.

We thank Mr. L. K. Sourirajulu, of the Zoology Department, for directing our attention to this animal in the net along with a collection of numerous other organisms. We also thank Mr. G. Ramakrishna, of the Zoological Survey of India, for help with a survey of literature. Our thanks are due to Prof. R. V. Seshaiya for his interest.

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LIMNOLOGY

A Lake in British Columbia containing Old Sea-Water

Stimulated by Strøm's reports[1,2], and by N. M. Carter's speculations (oral communication), we have made a preliminary examination of Powell Lake, British Columbia, and find conditions therein similar to those described by Strøm for Lakes Tokke and Botnvatn in Norway.

Powell Lake is a fjord lake about 50 km. long and 2 km. wide. The outline of the lake and the form of the mountains rising above it resemble the many fjord-like inlets along the British Columbian coast. However, the southern end of the lake (49° 53′ N., 124° 32′ W.) is separated from the adjacent

Strait of Georgia by a rocky sill 46 m. above sea-level.

A dam built fifty years ago downstream from the sill raises the lake-level to 56 m. above sea-level. Some 16 spot soundings made prior to our investigations indicated a maximum depth of 418 m., but the maximum which we recorded was 358 m. Of 10 soundings made by us in the southern part of the lake, a basin 10 km. long, 7 fell in the range 346–358 m. suggesting a nearly horizontal floor in this part. This lower part of the lake is separated from the upper part by two narrow straits which, judging from the few early soundings, are appreciably shallower than the basins above and below. The upper basins receive nearly all the important streams tributary to the lake. Turbidity currents which might develop directly from the muddy streams or by mud-slides from actively growing deltas are likely to be trapped in the upper basins, leaving the lower basin free, or nearly so, of invasions at depth by turbid water.

The salient water characteristics which make the Lake worthy of remark are shown in Fig. 1. Results were obtained on May 29 and 30, 1961, from the centre of the lower basin where the depths exceeded 350 m. The considerable rise in temperature of nearly 5° C. from 100 m. to the bottom was the first indication that the deeper water needed a significant salt content for stability. The chlorinity of the samples was determined by titration with silver nitrate and the salinity computed from Knudsen's relationship. It should be noted that this relationship is not entirely valid due to appreciable variations in the ratios of the ionic constituents of the water of the Lake from those found in modern sea-water. For example, the correction of the chlorinity titration for the presence of hydrogen sulphide (Table 1) reduces the salinity from 16·92 to 16·58 in the 334-m. sample. However, density and electrical conductivity values for the deep-water samples agreed closely with those for sea water of the same salinity. The presence of large amounts of dissolved gases in the bottom 50 m. of the water column was evident from the voluminous degassing which occurred while drawing the samples. These oxygen-depleted bottom waters had an *in situ* *p*H of 6·7–6·9 and showed a distinct yellow colour which increased in intensity with depth and was evidently due to dissolved organic matter. As shown in Table 1, the high hydrogen sulphide content of the bottom water results in the expected concurrent low iron (II) concentration. Curiously enough, this is a condition opposite to that found by Strøm[1] for Lake Tokke which in comparable chemical properties was otherwise similar to Powell Lake.

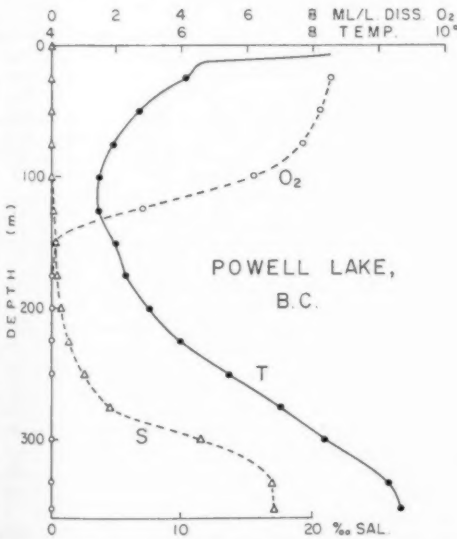

Fig. 1. Distribution of temperature, salinity and dissolved oxygen in Powell Lake, British Columbia

Table 1. Some Chemical Constituents of Powell Lake Bottom Water (334 m.)

Constituent	Concentration (mgm./l.)
Chloride	9,400
Sulphate	0
Bicarbonate	2,050
Total sulphide (H_2S, HS^-)	> 89
Total iron (dissolved and particulate)	< 0·5
Inorganic dissolved phosphate (P)	0·015
Inorganic dissolved silicate (Si)	10·4
Dissolved carbon dioxide	about 200
Dissolved methane	present *

* Identified by gas phase chromatography.

In the neighbouring inlets in which the stability of the water is high in the upper layers but decreases to the bottom a minimum temperature of 6°–8° C. is common at about 50 m. depth, while the deeper water is isothermal within 1° C. The minimum of lower value and greater depth in Powell Lake is attributed to the lower stability in the upper 150 m., which permits winter cooling to a greater depth. The considerable temperature gradient in the deeper water is associated with high stability of the deeper water due to the salinity gradient. It is possible that the temperature gradient is maintained by flow of heat through the bottom of the Lake. A flow of 40 cal./cm.²/yr. would require the eddy heat conductivity in the water to be 3–4 times the molecular conductivity.

The saline water is presumed to have entered the Powell Lake Valley first during the disappearance of the last Cordilleran ice sheet close to 13,000 years ago. At this period the land was submerged in the vicinity of the Strait of Georgia at least locally to the present 175 m. contour[3]. Quaternary marine fossils reported from one point on the east side of Powell Lake may belong to this stage. With continued uplift, during and following the disappearance of the ice sheet, the rocky sill at the lower end of the Powell Lake basin shoaled, became emergent, and gradually rose to its present position above the sea. Radiocarbon dates from the opposite side of the Strait of Georgia, 70 km. to the south[4], indicate that the sea stood at the present 52-m. contour about 12,250 years ago and there is no suggestion that this level was afterwards submerged. In the lower Fraser Valley, 180 km. south-east of Powell Lake, the sea withdrew from the 60-m. contour before the last ice disappeared, some time between 11,000 and 7,300 years ago. In the Fraser Canyon, 220 km. east of Powell Lake, the ice sheet had disappeared and the Fraser River flowed below the present 75-m. contour 9,150 years ago. At the stage when Powell Lake was cut off from the Strait of Georgia it is possible that the waters of the Strait had a salinity less than that of the present 30–31‰ due to dilution by large quantities of melt water from the dwindling ice tongues still present in parts of the lower Fraser valley[5,6] and at the head of at least one inlet[7] leading into the Strait.

We wish to acknowledge the assistance of Mr. J. Dice of Powell River, B.C., who made available and

operated his yacht, *Samarie*, from which our observations were made.

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HISTOLOGY

Use of Veronal Buffers in Formalin Fixatives

Formalin 'buffered' with veronal (diethylbarbituric acid) has been used as a tissue fixative by Zacks and Blumberg[1] and by Dales[2], and as a storage fluid for fixed and stained specimens by Essner *et al.*[3]. It does not appear to have been realized, however, that veronal reacts rapidly with formaldehyde to produce a substance that has no buffering capacity within the physiological range of *p*H values.

This was observed when the osmium tetroxide in Palade's veronal-acetate buffered fixative[4] was replaced by sufficient formaldehyde to give a final concentration of 4 per cent. The composition of the mixture was 2·6 ml. of analytical reagent quality 36 per cent formaldehyde, 5 ml. each of *M*/7 sodium veronal and *M*/7 sodium acetate, and 5·5 ml. of *N*/10 hydrochloric acid in a total volume of 23 ml. By analogy with the corresponding osmium tetroxide fixative, the formalin variant was expected to have a *p*H of about 7·2. In fact, the measured *p*H was 9·8, indicating that the formaldehyde had reacted with the sodium salt of the monobasic veronal[5] to produce a neutral product, with concomitant liberation of alkali.

The experiment was repeated several times under carefully controlled conditions, and the resulting solutions were titrated with *N*/10 hydrochloric acid, using a Radiometer (Copenhagen) automatic titrator Type *TTT*1 and recording titrigraph type *SBR*2. The results indicated that one molecule of alkali was produced for every molecule of sodium veronal undergoing reaction, and that all the veronal was converted into the reaction product in the presence of the excess formaldehyde in the mixture. The shape of the titration curve was almost indistinguishable from that of a solution containing the same concentrations of sodium acetate and formaldehyde as those in the fixative, but from which the veronal salt had been omitted. It is clear, therefore, that the reaction product of veronal and formaldehyde has no buffering capacity. Any residual buffering action in formalin variants of Palade's fixative is due solely to acetate and, consequently, effective only in the range of approximately 3·6–5·8.

The *p*H of veronal-treated formalin fixatives may, of course, be adjusted to any convenient value near neutrality. The solutions will still act as fixatives, for in the case described here, there is a concentration of free formaldehyde of about 3 per cent, which is adequate for many fixation purposes.

It is not clear why the effect described here has not been observed before. It is possible that the magnitude of the change in *p*H, and that of the adjustment necessary to restore the *p*H to the required level, has been overlooked through the use of inadequate detection methods. The effect is immediately obvious, however, when a sensitive *p*H-meter is used, and such instruments are essential for the preparation of buffered solutions for cytological and cytochemical purposes.

While the reaction of unsubstituted barbituric acid with formaldehyde is known to produce C,C′-methylene*bis*barbituric acid[6], the reaction with disubstituted barbituric acids does not appear to have been described before. Since the possibility of methylene bridge formation between carbon atoms is excluded by the presence of the two ethyl groups in veronal, the observed effect is probably due either to the formation of a neutral N-hydroxymethyl-derivative, or of the corresponding N,N′-methylene*bis*-compound. Structural investigations have not been made to confirm which of these suggestions is correct.

We have commented elsewhere[7] on the value, for electron microscopy, of formalin-based fixatives to which veronal and other buffers have been added.

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CYTOLOGY

Sub-Chromatid Structure and Chromosome Duplication in *Vicia faba*

A quadripartite structure of chromosomes has been inferred from certain anaphase configurations in mitosis and meiosis, such as the two-side-arm bridges found in irradiated meiosis in *Uvularia*[1] and *Lilium*[2]. Similar bridges have been seen in endosperm mitosis[3,4]. Wilson *et al.*[5] have clearly demonstrated the sub-chromatid nature of such bridges in *Trillium*.

The present work has aimed at determining whether sub-chromatids are demonstrable in *Vicia faba*, the species used by Taylor *et al.*[6] for autoradiographic analysis of chromosome duplication.

Meiotic material was fixed at half-hourly intervals 2–5 hr. after irradiation at a dosage of 36 r. Root tips (laterals), grown in distilled water, were fixed 1–1·5 hr. after 108 r. irradiation. Dosage-rate was 36 r./min. at 6 m.amp., 200 kV., with a 0·85 mm. copper/0·5 mm. aluminium filter. Focal distance was 40 cm., half-value layer 1·55 mm. copper. Timing trials indicated that anthers in mid- and late-first anaphase at fixation would have been in stages of late diplotene to metaphase 2–2·5 hr. previously. Divisions in control material were regular except for a very low frequency of breakage and reunion.

Pollen mother cells in first and second anaphase showed a high frequency of two-side-arm bridges similar to those described for *Trillium*[5] (Fig. 1a).

a *b*

Fig. 1. (*a*) Metacentric chromosome of *V. faba* at anaphase I showing true two-side-arm bridge. (× 1,900). (*b*) Anaphase I showing *Y*-shaped chromatid with corresponding deficient chromatid at opposite pole. (× 1,000)

It was possible to trace sub-chromatids in these configurations, and to distinguish 'true' and 'pseudo' bridging, resulting respectively from non-sister sub-chromatid reunion and from non-sister sub-chromatid reciprocal restitution. Configurations predicted but not seen by Wilson *et al.*, the one-side-arm bridge and the *Y*-shaped chromatid with a corresponding deficient chromatid at the opposite pole, were also found (Fig. 1*b*).

All these configurations, together with the whole chromatid bridges and fragments which also occurred, may be explained as consequences of different patterns of sub-chromatid reunion and restitution. Since most of the configurations require non-sister sub-chromatid breakage or even 3- or 4-strand breakage, it must be inferred that breakage involves the whole chromosome, while reunion is a function of the half-chromatids. The view that the chromosome is the functional unit in breakage is supported by the fact that in all bridges examined the side-arms appear to be of equal length; breakage must have been at homologous or near-homologous points on the chromatids.

Mitotic configurations were similar and included one-side-arm bridges and *Y*-shaped chromosomes. The two-side-arm bridges closely resembled the 'point unions' described by Davidson[7].

To distinguish between the sub-chromatid hypothesis of two-side-arm bridge formation and 'matrix stickiness' as suggested by Östergren and Wakonig[8], irradiated seedlings were transferred to 0·05 per cent colchicine for 24–30 hr. immediately after 108 r. irradiation. If sub-chromatids are concerned in bridge formation, whole chromatid errors should be evident in tetraploid *c*-metaphases. Sub-chromatid reunion, normally resulting in a true two-side-arm bridge, would yield a quadriradial X_2 association composed of a dicentric chromatid and an acentric fragment, while sub-chromatid restitution would yield a quadriradial of normal chromatids. Although only a small proportion of cells showed tetraploid X_2 mitoses after the colchicine treatment, both these configurations were found.

The minimal hypothesis of sub-chromatid structure is that each consists of a single deoxyribonucleic acid (DNA) double helix. Meselson and Stahl[9] have shown that, in replication of DNA, a first-generation molecule contains one original helix and a newly synthesized complementary helix. Taylor *et al.*[6] found a segregation of labelled and non-labelled chromatids in the X_2 *c*-metaphase, but with the existence of half-chromatids (with the minimal single DNA structure) such a situation should not be evident until the X_3 division. It has been shown[10] that there may be a substantial delay either in the penetration or the action of 0·05 per cent colchicine in *V. faba*. This could lead to a mis-identification of X_3 cells as being X_2, because of their tetraploidy.

Taylor's results would be compatible with a quadripartite chromosome, if segregation of labelled from non-labelled sub-chromatids should occur. Relational coiling of sister sub-chromatids would seem to prevent this: if, as seems likely on electron-microscope evidence, more orders of sub-units are present, this difficulty would be further increased. La Cour and Pelc[11] also have questioned the significance of Taylor's work after failing to obtain similar results in a repeat of the experiment: they suggest that colchicine may interfere with replication.

The apparent disagreement of the chromosome duplication studies with the demonstrable half-chromatid structure, as well as the lack of repeatability of the autoradiographic work, indicate that a thorough study of the techniques used in such work is needed before interpretations will be of significance.

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PSYCHIATRY

Barbiturates and Eye Vergence

It has been demonstrated[1] that barbiturates influence conjugate eye movements selectively in that they abolish the smooth tracking movements while leaving the saccadic movements intact. Disjunctive eye movements have time characteristics akin to the tracking movements; some effect of barbiturates on convergence has been mentioned[2].

In the following typical experiment a normal subject (age 21, height 6 ft. 3 in., weight 175 lb.) was given 390 mgm. amytal sodium ('Amobarbital Sod.', Lilly) by mouth. Measurements were taken, at suitable intervals over a 12-hr. period, of heterophoria with a target at 5 m., of the amplitudes of prism divergence and convergence with a target at

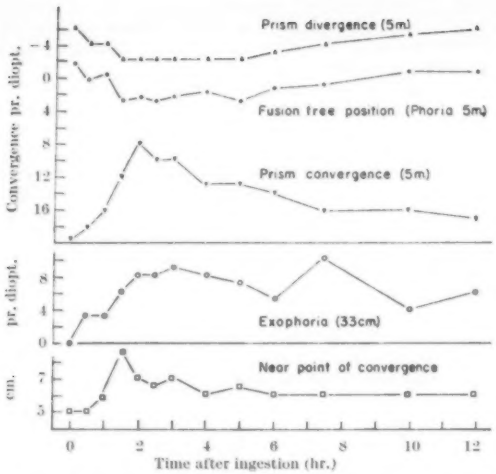

Fig. 1. Changes in a variety of vergence measures following ingestion of 390 mgm. amytal sodium

5 m., of heterophoria at 33 cm., and of the near point of convergence. The changes in the findings are illustrated in Fig. 1. The dose was moderate; during the phase in which he was most affected (2–6 hr. after ingestion) the subject's ability to make responses was unimpaired, he exhibited only mild barbiturate nystagmus on extreme version of the eyes, and some tracking eye movements persisted. Vestibular nystagmus was unimpaired. The effects illustrated in Fig. 1 were: a change in distance heterophoria in the direction of esophoria, a reduction in the amplitude of the fusional range, a marked change in the near heterophoria in the direction of exophoria, and a recession of the near point of convergence. Experiments with ethanol and anoxia indicate similar effects[2].

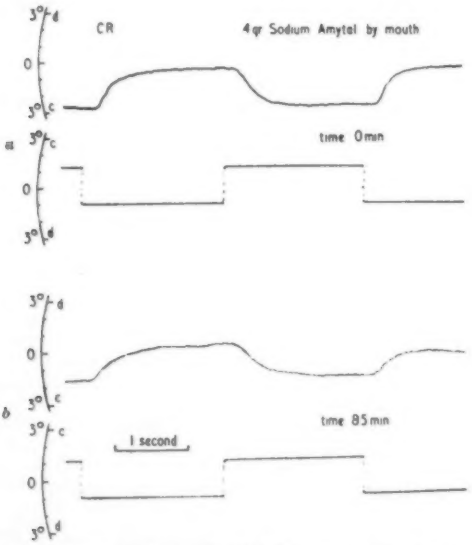

Fig. 2. Eye vergence response to 2° step change in target vergence. (*a*) Normal state; (*b*), 85 min. after ingestion of 260 mgm. amytal sodium

To establish whether or not those convergence responses that persisted in the limited fusional range were normal, eye movements records were obtained on one subject with a method described elsewhere[4]. The subject (age 33, height 5 ft. 7 in., weight 135 lb.) was given 260 mgm. amytal sodium by mouth and reached about the same level of intoxication as was demonstrated by the subject in the experiment in Fig. 1. In his normal state when the subject was presented with cathode-ray tubes at 2 m., one in front of each eye, on which spot targets were displaced suddenly so as to need a 2° vergence movement to re-establish binocular fixation, records such as those in Fig. 2*a* were obtained. During barbiturate intoxication the responses looked like those in Fig. 2*b*, demonstrating that, while the final level of convergence reached is the same as that in Fig. 2*a*, the time course of the response is slowed substantially. Records obtained from this subject during this phase showed that he still retains his capacity to make smooth pursuit eye movements during conjugate tracking of sine waves.

To determine whether the change of the heterophoria at near, which has considerable clinical implications in view of the relatively small dose of the drug involved, could be accounted for in part by a sensory change, we have also measured accommodation. If it does not suffer a deficiency similar to that of convergence, one cannot maintain that the effect of barbiturates is to inhibit in some way the total near response which involves both accommodation and convergence. Measurements of accommodation were obtained by three methods during some of these experiments: (1) the near point of accommodation, (2) the amount of accommodation in play while the subject was fixating the near heterophoria target, using retinoscopy, and (3) the amount of accommodation exerted by the subject during monocular view of a near point target through a semi-transparent mirror, using stigmatoscopy. In each case there was essentially no change in the accommodation measurements throughout the experiment. Neither was there any change in the refractive state for infinity, as determined by retinoscopy.

These findings show that barbiturates interfere with the mechanism which organizes eye vergence movements. This occurs with quite moderate clinical doses which barely affect smooth tracking movements. Saccadic and vestibular eye movements and accommodation responses remain intact under these conditions and this helps to delimit the site of action of the drug.

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APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RESEARCH FELLOW (with a good honours degree in a biological subject, some research experience in genetics or preferably cytogenetics, experience in the tissue culture field would be an advantage) TO WORK IN THE CYTOGENETICS GROUP of the Pædiatric Research Unit, Guy's Hospital Medical School—The Pædiatric Research Unit, Guy's Hospital Medical School, London Bridge, S.E.1 (August 25).

LECTURER (Grade II) or ASSISTANT LECTURER (Grade III) IN MYCOLOGY within the Department of Botany—The Registrar, The University, Bristol 8 (August 26).

SENIOR LECTURER (with a good honours degree, teaching experience, and either a higher degree or substantial research experience) IN THE DEPARTMENT OF CHEMISTRY, to be responsible for Physical Chemistry—The Principal, College of Further Education, Whitehaven, Cumberland (August 26).

DEMONSTRATOR IN PSYCHOLOGY, to assist in practical classes and tutorial work—The Registrar, The University, Liverpool, quoting Ref. CV/T (August 28).

GEOPHYSICIST (with a degree in science or electrical engineering, and with a knowledge of physics) in the South Australian Department of Mines—The Agent-General and Trade Commissioner for South Australia, South Australia House, Strand, London, W.C.2 (August 31).

LECTURER or SENIOR LECTURER (qualified to lecture up to honours standard) IN CHEMISTRY (ORGANIC or PHYSICAL) at Rhodes University, Grahamstown, South Africa—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, August 31).

LIBRARIAN (professionally qualified and experienced) at the Nigerian College of Arts, Science and Technology, Zaria—The Council for Overseas Colleges, 12 Lincoln's Inn Fields, London, W.C.2 (September 1).

PRINCIPAL LECTURER (preferably with an interest in the development of research projects) IN PHARMACEUTICS—The Principal, College of Technology, Belfast 1, Northern Ireland (September 1).

RESEARCH ASSISTANT (with a good honours degree in botany or agricultural botany and an interest in experimental ecology and/or genetics) IN AGRICULTURAL BOTANY, for investigations on the evolution and nature of resistance to zinc and copper toxicity in natural populations of higher plants—The Registrar, University College of North Wales, Bangor, North Wales (September 1).

RESEARCH ASSISTANT (with a qualification in chemistry and preferably previous experience in polymer chemistry) IN THE ISOTOPE UNIT, for work in the field of polymer reaction mechanisms—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (September 1).

ASSISTANT LIBRARIAN (university graduate, preferably with library qualifications)—The Librarian, University Library (University of St. Andrews), Dundee, Scotland (September 2).

LECTURER and an ASSISTANT LECTURER (graduates, with an interest in any of the branches of electrical engineering) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING of the Faculty of Science—The Registrar, The University, Manchester 12 (September 2).

EXPERIMENTAL OFFICER, Grade I (with a degree in physics or equivalent qualifications) at the Weapons Research Establishment, near Adelaide, Australia, to develop optical equipment required for field and laboratory performance testing of lens systems, and to carry out necessary performance testing—The Senior Representative (AV. 120/5), Department of Supply, Australia House, Strand, London, W.C.2 (September 4).

SENIOR LECTURER, Grade I or II (with a good honours degree and teaching experience, and preferably some research experience) IN MATHEMATICS at the Nigerian College of Arts Science and Technology, Zaria Branch—The Council for Overseas Colleges, 12 Lincoln's Inn Fields, London, W.C.2 (September 4).

LECTURER (preferably with some teaching experience and a veterinary qualification) IN THE DEPARTMENT OF VETERINARY PHYSIOLOGY, Royal (Dick) School of Veterinary Studies—The Secretary, The University, Edinburgh (September 5).

ASSISTANT CHIEF INSPECTOR OF NUCLEAR INSTALLATIONS (with a first- or second-class honours degree in engineering or physics, corporate membership of an appropriate professional institution, and wide experience of work on nuclear installations and the management of a team of professional engineers) in the Inspectorate of Nuclear Installations of the Ministry of Power to take charge of sections of the Inspectorate concerned with the safety assessment of nuclear installations, and with special problems in nuclear physics, chemistry, metallurgy and health physics—Civil Service Commission, 17 North Audley Street, London, W.1, quoting Ref. S/5355/61 (September 6).

ASSISTANT (graduate in botany or agriculture or equivalent qualifications) TO THE MYCOLOGIST, for work involving testing the susceptibility to disease of crop varieties, and at present is mainly concerned with the cereals—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge (September 15).

LECTURER and a SENIOR LECTURER IN THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, Monash University, Clayton, Victoria, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, September 15).

DIRECTOR OF COMBINED STUDIES IN THE FACULTY OF SCIENCE, to supervise admissions to, and the progress of, students in the courses leading to the newly instituted degree in which two science subjects are studied to a higher level—The Registrar, The University, Leeds 2 (September 18).

SENIOR ASSISTANT CHEMIST (member of an appropriate chemical institution, extensive experience in large-scale sewage purification and trade effluent control, and preferably with a university degree and membership of the Institute of Sewage Purification) at Mogden Purification Works, Isleworth, Middlesex—The Chief Chemist, Middlesex County Main Drainage Department, P.O. Box No. 7, Isleworth, Middlesex, quoting H.23T (September 18).

SENIOR RESEARCH FELLOW or RESEARCH FELLOW (with a Ph.D. degree in organic chemistry, with additional experience in general synthetic organic chemistry) IN THE BIOLOGICAL INORGANIC CHEMISTRY UNIT, John Curtin School of Medical Research, Institute of Advanced Studies, Australian National University, to work on the synthesis of multidentate organic ligands, as part of the programme of the Unit on the effects and utilization of metal complexes in biological systems—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 18).

UNIVERSITY DEMONSTRATOR (with some acquaintance with population studies and biological statistics) IN PHYSICAL ANTHROPOLOGY—The Secretary of the Appointments Committee, Faculty of Archæology and Anthropology, The University, Downing Street, Cambridge (September 18).

SENIOR LECTURER or a LECTURER IN THE DEPARTMENT OF ELECTRICAL ENGINEERING at Newcastle University College, University of New South Wales—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2, and the Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (September 22).

EXPERIMENTAL OFFICER or SENIOR EXPERIMENTAL OFFICER (preferably with previous experience of high voltage engineering, electronics, and nuclear physics, and a degree or diploma in physics or electrical engineering), to take charge of the maintenance and operation of the Manchester University 6-MeV. electrostatic generator—The Registrar, The University, Manchester 13 (September 30).

FULL-TIME LECTURER or SENIOR LECTURER IN PATHOLOGY—The Director, Department of Pathology, Institute of Ophthalmology (University of London), Judd Street, London, W.C.1 (September 30).

LECTURER IN THEORETICAL PHYSICS; a LECTURER IN PHYSICS; and ASSISTANT LECTURERS IN PHYSICS—The Registrar, The University, Manchester 13 (September 30).

NUFFIELD FOUNDATION RESEARCH FELLOWS (2) (well qualified in electrical engineering or physics with general experience in electronics and considerable detailed knowledge of solid state circuits) IN THE SCHOOL OF ELECTRICAL ENGINEERING, University of Sydney, Australia, to be associated with the development of electrical engineering aspects of the design of a large radioastronomy interferometer system under development by the Radio Astronomy Centre—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (September 30).

SENIOR DEMONSTRATOR (graduate) IN ZOOLOGY at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

LECTURER IN APPLIED PATHOLOGY—The Secretary, Queen's University, Belfast, Northern Ireland (October 1).

LECTURER/SENIOR LECTURER IN BOTANY at the University of New England, Armidale, New South Wales, Australia, to lecture in plant physiology or in mycology-microbiology to advanced students in botany and to supervise research projects of postgraduate and final year (botany double major) students—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 6).

ASSISTANT EXPERIMENTAL OFFICER (with H.N.C. or pass degree, or, if under 22, G.C.E. "A" level or equivalent) IN THE PHYSICS DEPARTMENT, to assist in studies of physical properties of dairy products—butter, cheese, etc.—and to construct and modify experimental testing equipment in the departmental workshop—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 61/4.

BIOCHEMIST or CHEMIST, to study metabolism of ^{14}C-labelled steroid hormones—The Professor of Physiology, The Medical School, King's College (University of Durham), Newcastle-upon-Tyne 1.

BIOCHEMIST (qualified), for steroid work in an endocrine laboratory in the Obstetric Unit of the Medical School of University College Hospital—The Secretary, University College Hospital Medical School, University Street, London, W.C.1.

GRADE B ASSISTANT TO TEACH PHYSICS—The Principal, Huddersfield College of Technology, Huddersfield.

GRADUATE ASSISTANT (science graduate, preferably with some dairy experience), to undertake a study of pipeline milking—Head of the Department of Dairy Science, University of Alberta, Edmonton, Alberta, Canada.

GRADUATE or TECHNICIAN (A.M.I.L.T.), to assist with research on problems of fibrinolysis in relation to coronary thrombosis, etc., the work will be mainly hæmatological—The Assistant Secretary, Westminster Hospital, London, S.W.1.

NUFFIELD RESEARCH ASSISTANT (with a good honours degree in either physics or a biological science) IN BIOPHYSICS, to work under the direction of Dr. K. W. Keohane—The Secretary, Chelsea College of Science and Technology, Manresa Road, London, S.W.3.

POSTGRADUATE STUDENT (man or woman graduate with an honours degree in chemistry), for work involving research in boron chemistry—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey.

RESEARCH ASSISTANT, to help with current research on an immunological project connected with rheumatoid arthritis—The Pathologist, Stoke Mandeville Hospital, Aylesbury, Bucks.

RESEARCH ASSISTANT, to undertake programming investigation in connexion with a second college digital computer now under construction—The Principal, College of Technology, Broadway, Letchworth, Herts.

RESEARCH ASSISTANT (with a B.Sc. degree) IN THE DEPARTMENT OF MEDICINE, to join a small team working in cardio-renal physiology in its relation to human disease—Joint Clerk to the University Court, Queen's College (University of St. Andrews), Dundee.

RESEARCH STUDENT (with an honours degree or equivalent qualification, for example, G.R.I.C.) IN ELECTRODIALYSIS, to carry out research for a period of three years under the direction of Dr. P. Meares on fundamental aspects of the transport of ions in charged membranes using radioactive tracers—Prof. G. M. Burnett, Department of Chemistry, The University, Old Aberdeen.

RESEARCH TECHNICIAN (with some experience in a biochemical research laboratory), for work in the Department of Chemistry and Food Technology on chemical and fermentation studies of the cocoa

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bean—The Secretary, Borough Polytechnic, Borough Road, London, S.E.1.

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Technical Officer (Utilization) (with a Diploma or City and Guilds Final Certificate in machine wood-working, wood technology or allied subject) in the Department of Forest Research, Nigeria, to take charge of one or more sections of the new Forest Products Research Laboratory, including wood-working, seasoning, impregnation and timber mechanics—The Recruitment Attaché, Nigeria High Commission, 9 Northumberland Avenue, London, W.C.2, quoting Ref. 1.2/3.

Technician (male or female) in the Microbiological Teaching Laboratory—Mr. Edwards, The School of Pharmacy, 29/39 Brunswick Square, London, W.C.1.

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